Role of homocysteine
in
Proliferative diabetic retinopathy:
a case-control study

CHRISTIAN MEDICAL COLLEGE
VELLORE
INDIA

DISSERTATION SUBMITTED TOWARDS FULFILLMENT OF THE RULES
AND REGULATIONS FOR THE M.S. BRANCH III OPHTHALMOLOGY
EXAMINATION OF THE TAMILNADU

DR. M.G.R. MEDICAL UNIVERSITY

TO BE HELD IN APRIL, 2016

Role of homocysteine in Proliferative diabetic retinopathy: a case-control study

SUBMITTED BY

Dr. PRABHA GUPTA

CHRISTIAN MEDICAL COLLEGE

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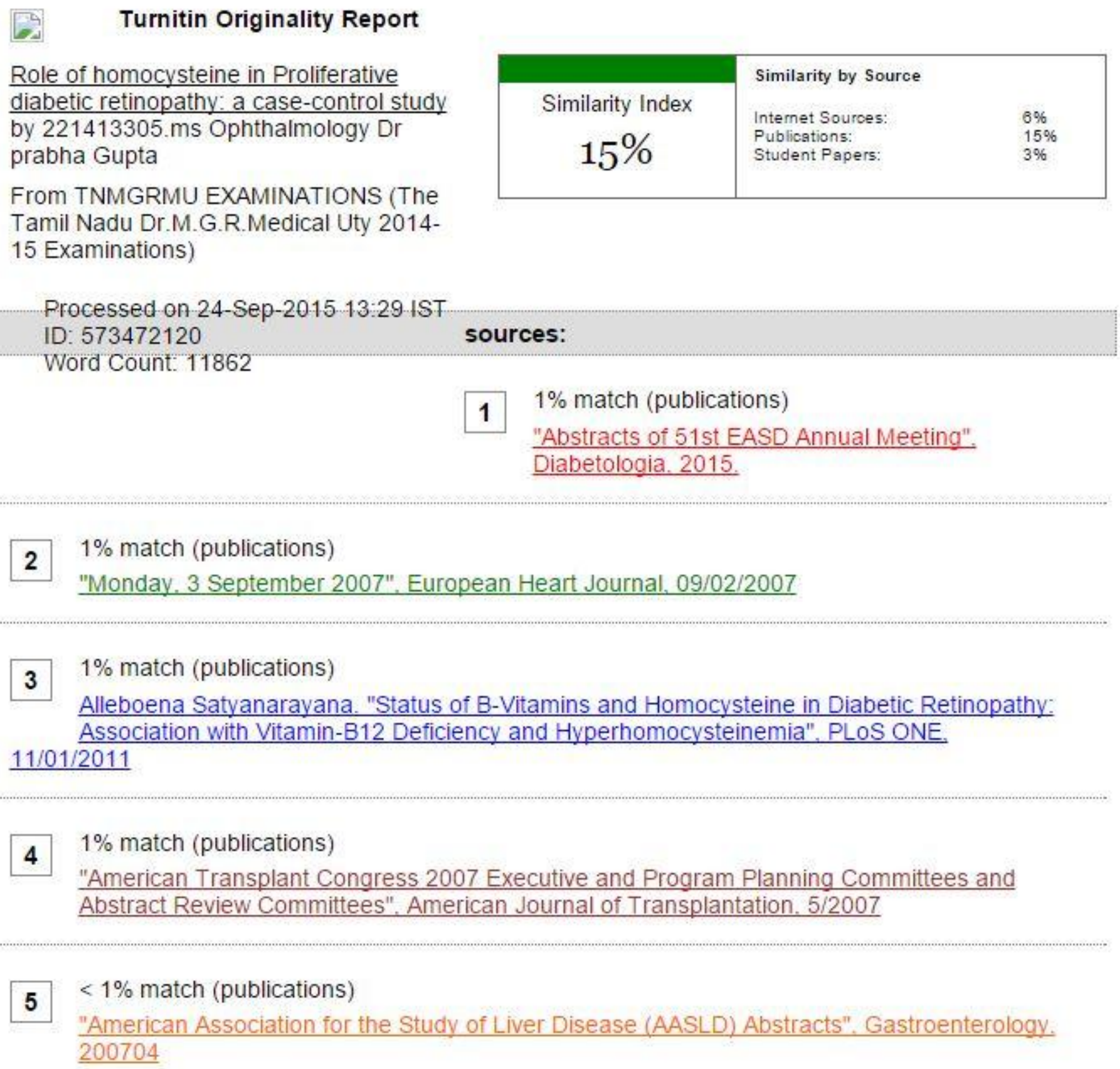

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INTRODUCTION

Diabetes mellitus (DM) refers to a spectrum of metabolic disorder that has hyperglycemia as its common phenotype. Different types of diabetes mellitus (DM Type 1 and DM Type 2) are caused by interaction of several environmental and genetic factors. Diabetic retinopathy (DR) is one of the highly specific vascular complications of diabetes mellitus.(1)

Diabetic retinopathy is predominantly a microangiopathy, in which the small blood vessels of the retina are damaged due to hyperglycemia. This results in capillary leakage and non-perfusion, leading to retinal edema and hypoxia. Retinal hypoxia and ischemia lead to neovascularization, which is diagnostic of proliferative diabetic retinopathy (PDR). This subsequently results in fibrovascular proliferation, vitreous hemorrhage and tractional retinal detachment, leading to severe, often irreversible visual impairment and blindness.(2)

Globally, there are 382 million people diagnosed to have diabetes; by 2035, this number will increase to 592 million.(3) Globally, DR accounts for approximately 4.8% cases of blindness as per Who estimates.(4) DR is also a leading cause of new-onset blindness in working age population in industrialized countries, and an increasingly frequent cause of blindness in low -middle income countries.

Presently, India is home to 65.1 million people living with diabetes, and the figure may reach to estimated 109 million people with diabetes living in India by the year 2035.(3) Diabetes is a major public health burden, adding to the economic woes of a developing country like India. The prevalence of DR, as estimated in the CURES Eye study, was 17.6% among diabetic patients.(5) This indicates the magnitude of the epidemic, which leads to a huge strain on the health expenditure of a developing country. In India, DR is becoming a leading cause of visual impairment due to several fold increase in diabetic population, as all patients with diabetics will develop some form of DR within 20 years of disease onset.(6) At present, there are not many measures to prevent the onset of DR. However, there are various known risk factors which affect the disease progression like duration of diabetes, poor glycemic control, pregnancy, hypertension, nephropathy, hyperlipidemia and anemia. Several large, multicentric studies have shown that control of systemic disease can delay the onset of retinopathy and slow down its progression.(7–9) Its, important to mention, that despite a good glycemic control, some patient may go on to develop diabetic retinopathy. Conversely, few patients with poor glycemic control may escape this complication.

Therefore, multiple factors are likely to be involved in predisposing diabetic patients to develop retinopathy. If the factors predisposing to develop retinopathy are known, it may be possible to delay its onset and progression. Hence, there is an urgent and obvious need to understand and delineate the several risk factors which are associated with development and progression of diabetic retinopathy. Of the various risk factors that are hitherto known to cause progression of retinopathy, some are modifiable. Hyperglycemia, hypertension, anemia, hyperlipidemia, obesity and nephropathy are modifiable to varying extents. Non-modifiable risk factors are age, duration of diabetes and genetic predisposing factors. Pregnancy has been identified to be an independent risk factor for progression of retinopathy.(10)

In recent years, hyperhomocysteinemia has been postulated as a potential risk factor for development and progression of retinopathy in patients with diabetes. Homocysteine (Hcy), an intermediate molecule in methonine metabolism, has generated interest in recent years as a risk factor for cardiovascular disease and other vaso-occlusive diseases, including retinal vessel occlusion.(11) Higher blood levels of homocysteine are considered toxic to the vascular endothelium through generation of free radicals. Free radicals cause disruption of endothelial integrity, leading to platelet activation, causing hypercoagulability and thrombus formation.(12)

Several studies have been done worldwide to investigate the role of hyperhomocysteinemia in DR. Some of these studies have concluded that hyperhomocysteinemia is associated with increased risk for development and progression of DR.(11–14) However, certain studies have not found an association between hyperhomocysteinemia and diabetic retinopathy.(15,16) Therefore, there has been no definite evidence so far, to prove or disprove the role of hyperhomocysteinemia as an independent risk factor for development and progression of diabetic retinopathy. Moreover, different studies have used different cut off values to define hyperhomocysteinemia, ranging from 12 μmol/L to 15 μmol/L.(11-12,14) Deficiency of vitamin B12 and folate has been associated with increased serum homocysteine levels. Hyperhomocysteinemia could therefore, be a potentially modifiable risk factor for diabetic retinopathy. Dietary supplementation could be achieved at a very affordable cost, thereby saving the patient not only from the burden of morbidity caused by the disease, but also from the economic impact of the medical expenses incurred. This is especially relevant in India, where there is a high prevalence of diabetes as well as vitamin B12 deficiency.(17–19) Hence, understanding and characterizing the role of hyperhomocysteinemia in the pathogenesis of DR may help in identifying a novel target to combat this potentially blinding disease.

The aim of this study was to determine the role of hyperhomocysteinemia in proliferative diabetic retinopathy.

AIM AND OBJECTIVE

Aim:

To determine the role of hyperhomocysteinemia in proliferative diabetic retinopathy

Objective:

To determine the association of hyperhomocysteinemia with proliferative diabetic retinopathy

REVIEW OF LITERATURE

The term 'Diabetes mellitus' refers to a group of metabolic disorders characterized by impaired glucose metabolism, either due to deficiency of insulin or its resistance, resulting in hyperglycemia. This hyperglycemia in turn may result in development and progression of multiple vascular and neuropathic complications in patients with diabetes.

There are two main types of diabetes mellitus:

Type 1(Insulin dependent diabetes mellitus):

There is autoimmune destruction of pancreatic β-cells, resulting in absolute deficiency of insulin in patients with Type 1 diabetes mellitus (T1DM).

Type 2 (Non-insulin dependent diabetes mellitus):

There is decreased sensitivity of target tissues to insulin, leading to insulin resistance or relative insulin deficiency in patients with Type 2 diabetes mellitus (T2DM).

Insulin deficiency affects glucose metabolism by preventing the efficient uptake and utilization of glucose by most cells of the body, leading to hyperglycemia. The common metabolic dysregulation associated with diabetes mellitus (DM) causes secondary pathophysiologic imbalance in multiple organ systems, imposing huge burden on the patients itself and also on the health care system.(20,21)

The complications of DM can be classified into:

1. Acute complications
    a. Diabetic Ketoacidosis (DKA)
    b. Hyperglycemia
    c. Hyperosmolar state
2. Chronic Complications
    a. Microvascular Complications
        i. Retinopathy
        ii. Neuropathy
        iii. Nephropathy
    b. Macrovascular complications
        i. Coronary heart disease
        ii. Peripheral arterial disease
        iii. Cerebrovascular disease
    c. Others
        i. Gastrointestinal (gastroparesis and diarrhea)
        ii. Genitourinary (sexual dysfunction and uropathy)
        iii. Dermatologic complications
        iv. Increased susceptibity to infection
        v. Periodontal disease
        vi. Hearing loss
        vii. Visual loss (cataract)

The risk of chronic complications associated with DM, increases as a function of duration of the disease and severity of hyperglycemia. These complications usually do not become clinically apparent until the 2nd decade of hyperglycemia. Hence, the duration of diabetes plays a major role in the development and progression of complications in patients with T1DM. Similar results have been obtained in patients with T2DM. However, in T2DM, the time of onset, and therefore the duration, has been more difficult to determine precisely. So the individuals with newly diagnosed T2DM occasionally present with complications such as diabetic retinopathy as their initial sign of presentation, or are found to have retinopathy soon after the diagnosis of their systemic disease.(22)

Diabetic retinopathy

Diabetes mellitus can affect both the neuronal and vascular components of the retina. DR is a vascular complication of retina, common to both T1DM and T2DM. It is predominantly a microangiopathy, in which the small blood vessels of the retina are damaged due to hyperglycemia. It comprises of cellular damage and capillaropathy, which includes loss of pericytes, thickening of basement membrane and endothelial cell proliferation.

The probable pathophysiologic mechanism by which hyperglycemia causes vascular disruption, is by the formation of reactive oxygen species (ROS), leading to a cascade of biochemical changes,(20) which includes: -

(1) Increased activation of protein kinase C, leading to:

  a) Increased permeability of retinal vasculature and altered retinal blood flow

  b) Thickening of basement membrane leading to ischemia

  c) Cellular signalling through vascular endothelial growth factors causing retinal neovascularization

(2) Accumulation of advanced glycation end products

(3) Polyol (such as sorbitol) accumulation - Hyperglycemia causes increased metabolic conversion of glucose into sorbitol. This increase in intracellular sorbitol concentration causes osmotic damage to retinal vasculature.

(4) Oxidative stress – due to formation of free radicals along with the above mentioned biochemical pathways leads to retinal vasculature damage.(20)

These changes result in two basic pathological changes in the retina: retinal capillary non perfusion and retinal capillary leakage. The changes seen in the early stages of non-proliferative diabetic retinopathy, such as microaneurysms, hemorrhages and hard exudates result from retinal capillary leakage. Retinal capillary non-perfusion leads to retinal ischemia, hypoxia, intraretinal microvascular abnormalities and neovascularization. Neovascularization is diagnostic of proliferative diabetic retinopathy.(20)

Clinically evident signs of diabetic retinopathy

The first clinically evident signs of diabetic retinopathy are the subtle changes in the retinal vasculature - retinal ‘microaneurysms’. Microaneurysms usually develop in the area of compromised vascularity, as saccular outpouchings of retinal capillaries in the inner nuclear plexus, due to weakening of the capillary wall and endothelial cell proliferation. Deep hemorrhages present in DR are either small dots or larger blot haemorrhages arising from capillaries in the deeper layers of the retina.

Superficial hemorrhages are flame-shaped hemorrhages arising from capillaries in the nerve fibre layer. Hard exudates are the deposition of lipoproteins from the leaking capillaries, arranged in a circinate configuration around leaking micro-aneurysms or leaking retinal capillaries. Cotton wool spots (soft exudates) result from ischemia of the retinal nerve fibre layer, and are present at the junction of ischemic and non-ischemic retina. They present as white fluffy lesions with ill defined margins. Areas of venous changes present in diabetic retinopathy (beading and looping) correlate with the probability of progression to diabetic retinopathy. Intra retinal micro-vascular abnormalities (IRMA) represent pre-existing vessels with endothelial cell proliferation that have become ‘shunts’ that run from retinal arterioles to venules to bypass the capillary bed in areas of capillary non perfusion. Multiple IRMAs indicate a severe stage of non-proliferative retinopathy with a higher risk of development of frank neovascularization. Retinal neovascularization commonly occurs in areas of previously existing IRMA. Arteriolar changes present in diabetic retinopathy range from mild arteriolar dilation in early stages, followed by peripheral narrowing and obliteration, suggestive of vascular occlusion.

PDR is characterized by the development of retinal neovascularisation. This is most often seen at or near the optic disc (NVD), or elsewhere in the retina (NVE), and proliferates on the posterior vitreous face. This can progress to form fibrovascular proliferation, vitreous hemorrhage and tractional retinal detachment.(2,20)

Factors influencing diabetic retinopathy

Duration of diabetes, poor metabolic control, hypertension, nephropathy, pregnancy, abdominal obesity, hypercholesterolemia, dyslipidemia, anemia, cardiovascular and peripheral autonomic neuropathies are associated with the presence of DR. The two major risk factors for DR are the duration of diabetes and poor metabolic control. According to the Wisconsin Epidemiological Study of Diabetic Retinopathy, nearly all patients with T2DM, and about 60 % of patients with T1DM developed some form of DR after twenty years of disease.(22,23) According to the UK Prospective Diabetes Study, intensive glycemic control can delay the onset and progression of diabetic retinopathy.(8–9) The Diabetes Control and Complications Trial proved that a 10 % reduction in glycosylated hemoglobin level can significantly reduce the risk of onset, and retard the progression of retinopathy.(24) This study also found that intensive control of blood sugar reduced the risk of developing DR by nearly 76%, and retarded the progression by nearly 54% in patients having retinopathy. Several studies have also impressed upon the strict control of blood pressure, blood glucose, and hyperlipidemia control in preventing the complications in patients with T1DM and T2DM. (25–28)

Classification of diabetic retinopathy

The most commonly used classification of DR is the Modified Airlie House Classification, which was introduced by the Early Treatment Diabetic Retinopathy Study (ETDRS).(2) Diabetic macular edema is an important determinant of visual function in diabetics. Diabetic macular edema can be present either with non-proliferative or proliferative diabetic retinopathy.

Modified Airlie House classification (2)

1. Non proliferative Diabetic Retinopathy

A. Mild NPDR

Presence of at least 1 microaneurysm

Definition not met for B, C, D, E, F

B. Moderate NPDR

Hemorrhage (H)/ Microaneurysm (Ma) ≥ standard photograph no. 2A

Soft exudates, venous beading (VB) & IRMA definitely present

Definition not met for C, D, E, F

C. Severe NPDR

H/Ma ≥standard photograph No.2A in all 4 quadrants

VB in ≥ 2 quadrants

IRMA > standard photograph No. 8A in at least 1 quadrant

D. Very Severe NPDR

Any 2 or more of C

Definition not met for E, F

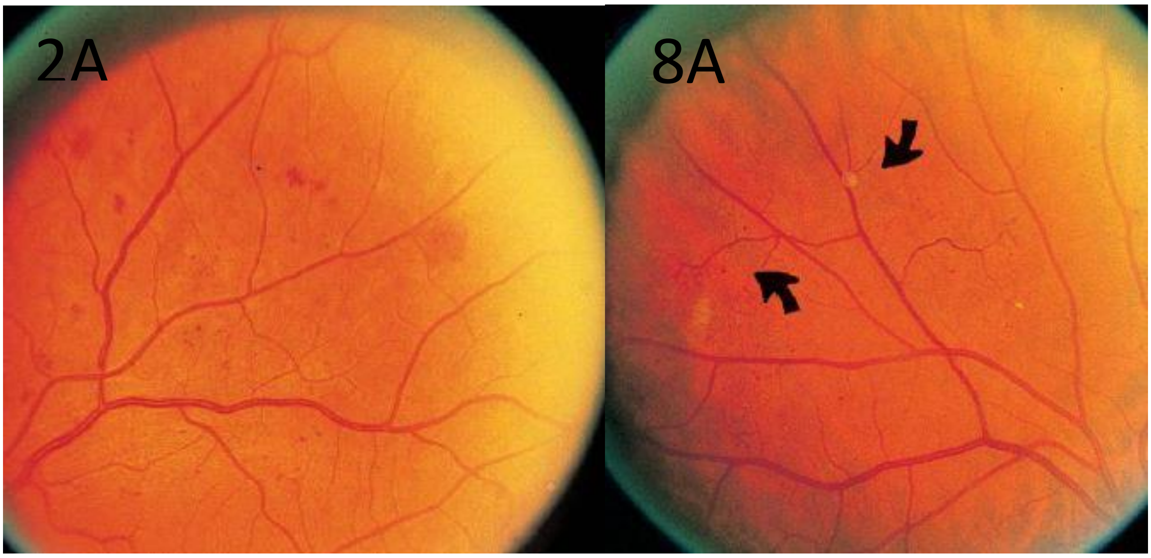


Figure 1 showing Standard photographs 2A and 8A of the Modified Airlie House classification

2. Proliferative Diabetic Retinopathy (PDR)

PDR is characterized by the presence of neovascularization either at the disc or somewhere on the retina other than the disc, which may produce complications like vitreous hemorrhage or preretinal haemorrhage, and fibrovascular proliferation.

E. Early PDR

Formation of new vessels

Definition not met for F

F. High Risk PDR

NVD (1/3-1/2 disc area)

NVD and Vitreous or preretinal hemorrhage

NVE ≥ ½ disc area and Vitreous or preretinal hemorrhage

3. Clinically Significant Macular Edema (CSME) [any one of the following criteria]

1. Retinal thickening at or within 500 microns from the centre of the macula, or
2. Presence of hard exudates with adjacent retinal thickening located at or within 500 microns from the centre of the macula, or
3. A zone of retinal thickening which is more than or equal to 1 disc area in size, located at or within 1 disc diameter from the centre of macula

Blindness due to diabetic retinopathy

WHO has estimated that 4.8% of the blindness throughout the world is due to DR. (4) DR has emerged as a leading cause of new onset blindness especially in working age population of industrialized world, and as a frequent cause of blindness in developing middle income countries.

Avoidable blindness

Avoidable blindness is defined as visual loss, which could be either treated or prevented by cost-effective measures. Nearly 75% of the world's blindness is avoidable, and diabetes contributes to about 5%.(29) Vision 2020 aims to address the major causes of avoidable blindness, in order to produce the largest impact on vision loss across the globe. Among these, cataract is the leading cause of avoidable blindness, and diabetic retinopathy (tenth on the list) is currently not the primary cause of avoidable blindness.(30) However, it has the potential to become the leading cause of blindness in 20 years time, and will affect the poorest people the most, as 80% of people with DM currently resides in low and middle income countries.(31)

Diabetic retinopathy: prevention

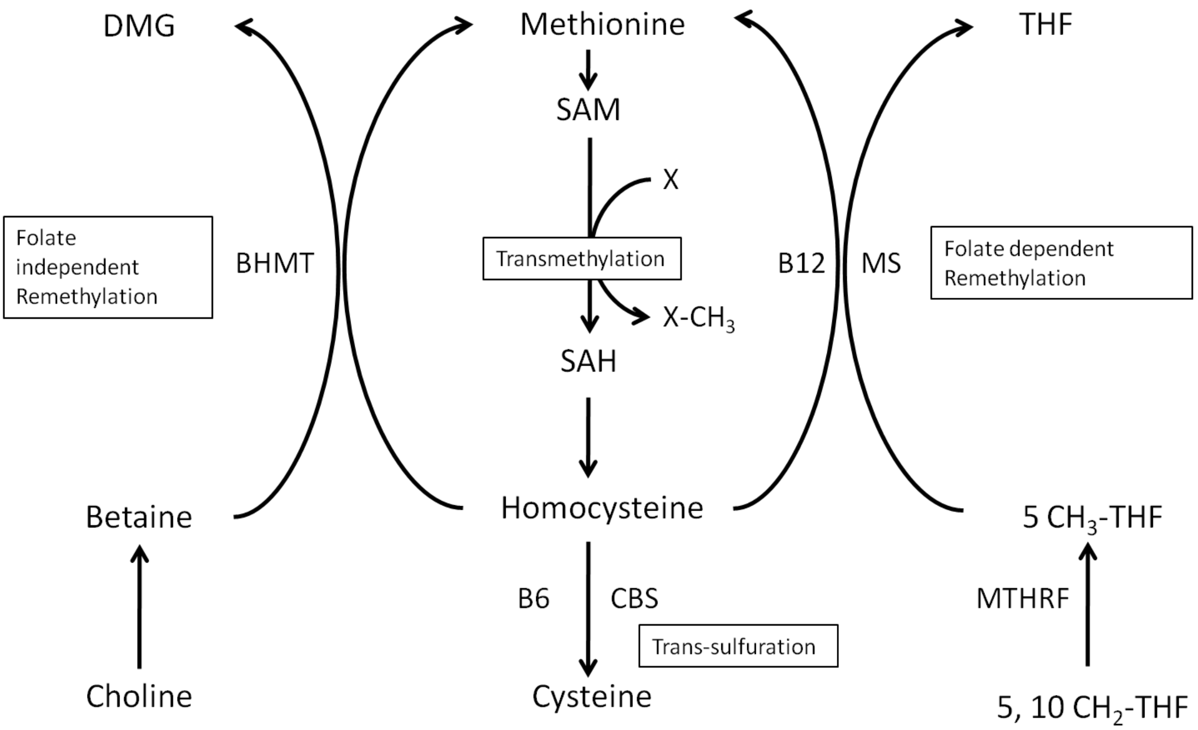
At the present time, there are no known means to completely prevent the onset of DR. However, evidence from several large, multi-centric clinical trials supports the beneficial role of adequate control of systemic disease and other co-morbidities in delaying the onset and progression of disease.(22,32,33)

Hyperhomocysteinemia - an emerging risk factor for progression of DR

Homocysteine (Hcy) is a methylated derivative of methionine, which is formed during methionine metabolism. Any abnormality in the metabolism of homocysteine can lead to hyperhomocysteinemia. Methionine is converted into S-adenosylmethionine (SAM), which is an important methyl group donor in the body. After donating the methyl group, methonine is converted to homocysteine. The homocysteine formed in this way is metabolized by two different pathways:

1) **Remethylation** – Hcy forms methionine by addition of a CH_3- group from 5-methyltetrahydrofolate (5 MTHF) or betaine. This 5 MTHF is generated from folic acid in the presence of the enzyme, 5-10-methyltetrahydrofolate reductase (MTHFR), and cofactor vitamin B12. Betaine pathway is independent of folate metabolism.

2) Trans-sulfuration - In the trans-sulfuration pathway, Cystathionine is formed from Hcy by the enzyme cystathionine β-synthase, and finally it is converted to cysteine, using pyridoxine as a cofactor.(34)



DMG: Dimethyl glycine, BHMT: Betaine homocysteine methyl transferase, SAM: S adenosyl methionine, SAH: S adenosyl homocysteine, THF: Tetrahydrofolate, MTHRF: Methyl Tetra hydrofolate reductase, MS: Methionine synthase, B6 : Vitamin B6, B12: Vitamin B12

Figure 2 showing metabolism of homocysteine

Severe hyperhomocysteinemia can be due to rare genetic defects, which result in deficiencies in methyltetrahydrofolate reductase, cystathionine β-synthase, or in enzymes involved in the methylation of homocysteine and methylcobalamine synthesis.(35) Mild hyperhomocysteinemia seen in fasting conditions, is due to vitamin B12 or folate deficiencies, or thermolability of methyl-tetrahydrofolate reductase, which result in a mild impairment of the methylation pathway.

The most common cause of genetic hyperhomocysteinemia results from production of a thermolabile variant of MTHFR with reduced enzymatic activity (T mutation).(36) Homocysteine concentration is affected by a number of factors, some of which are modifiable, like nutritional status, lifestyle and disease processes. Non-modifiable factors include age, gender and ethnicity. Homocysteine concentration in serum increases throughout life, and becomes approximately double that of childhood in old age. Men have higher serum concentration of homocysteine compared to women, with a mean difference of 2 µmol/L. This difference decreases with age; however, above a given upper reference limit, there is no difference.(37,38) Plasma levels of homocysteine increase from 10.8 µmol/L at age 40–42 years, up to 12.4 µmol/L between 65- 67 years.

Hyperhomocysteinemia has been classified as moderate (15–30 µmol/L), intermediate (31–100 µmol/L) or severe (>100 µmol/L) based on serum Hcy levels.(38) The mean serum concentrations and upper reference limits of homocysteine are markedly reduced in physiological states like pregnancy.(39,40)

In the last few years, numerous studies have been done to study the direct or indirect influence of elevated serum Hcy levels in several conditions, including vascular and neurodegenerative diseases **(**Alzheimer's disease & Vascular dementia), auto immune disorders, birth defects (neural tube defect, non-syndromic oral cleft, congenital heart defect), DM, osteoporosis, renal disease, neuropsychiatric illness (depression) & cancer (breast cancer) . Various studies have shown hyperhomocysteinemia as an independent risk factor for vascular disease like atherosclerosis and coronary artery disease.(34)

Pathophysiology of hyperhomocysteinemia in diabetic retinopathy

Elevated serum Homocysteine level is an emerging risk factor for several vascular occlusive diseases like coronary artery disease, cerebrovascular accident and deep vein thrombosis. It is also considered a risk factor for ocular vascular occlusive diseases like retinal venous occlusion, retinal arterial occlusion, ischemic optic neuropathy and diabetic retinopathy.

Figure 3: Flow diagram showing pathophysiology of hyperhomocysteinemia affecting microvasculature in diabetic retinopathy

Hyperhomocysteinemia with diabetes
↓
↑ oxidative stress & generation of free radicals
↓
Disruption of endothelial integrity
↓
Exposure of vascular matrix and underlying smooth muscle cells
↓
Promotion of hypercoagulable state
↓
Thrombus generation
↓
Further vascular compromise
↓
Increase in capillary drop out
↓
Further increase in hypoxic ischemia of retina in diabetic retinopathy(12,13)

Recent studies indicate that methylation of DNA is essential for both DNA repair and gene stability. There is a growing body of evidence which suggests that histone modification and DNA methylation plays a crucial role in the development of DR. The inactivation of DNA repair pathways leads to chromosomal instability an increased mutation rate, which can initiate and accelerate the proliferative process. The increased proliferation rate of cells would cause an elevation of Hcy levels in blood, or an increase in the concentration of cells would deplete folate and inactivate the methionine synthase-catalyzed remethylation reaction. This potential link between the microvascular changes that occur in DR and hyperhomocysteinemia may be useful as a predictor for retinopathy. The link between elevated homocysteine levels and microvascular changes could potentially be used as an early predictor of DR in patients with DM, and this could facilitate optimal management of retinopathy well in advance, before the occurrence of blinding complications.(12,13)

Factors affecting homocysteine levels in blood

The homocysteine levels in blood are determined by various factors, which include genetic factors, lifestyle factors, physiologic factors and various clinical conditions and drugs.

Important factors are listed below:(37)

Genetic factors:

Homocystinuria, Down syndrome, MTHFR 6777C→T polymorphism

Physiological factors:

Plasma homocysteine concentration increases with increasing age, male gender, increasing muscle mass, reduced glomerular filtration rate, postmenopausal state and decreases during pregnancy.

Life style factors:

Plasma homocysteine concentration increases with smoking, alcohol consumption, coffee drinking and decreases with vitamin supplementation.

Clinical conditions:

Folate / vitamin B12 / vitamin B6 deficiency, renal failure, hyperproliferative disorders and hypothyroidism are associated with increased concentration of homocysteine, while hyperthyroidism is associated with decrease in homocysteine concentration.

Drugs:

Drugs associated with increased homocysteine concentration are:

- Methotrexate and Trimethoprim by inhibiting DHFR
- Cholestyramine by inhibiting folate absorption
- Metformin, H2 receptor antagonists and Proton pump inhibitors by inhibiting cobalamine absorption
- Niacin, Azauridine, Theophylline by inhibiting pyridoxal kinase.
- Other drugs: Fibrates, Diuretics, Cyclosporin A

Drugs associated with decreased homocysteine concentration are: D-Penicillamine, N-Acetylcysteine, Estrogen, Tamoxifen, Betaine, Simvastatin.

Methods of homocysteine estimation

There are three available methods for homocysteine estimation:

- Immunoassay method
- Chromatographic method/ HPLC method
- Enzyme cycling method

The first step is the conversion of different species of homocysteine into the reduced form, which is then measured by different methods.

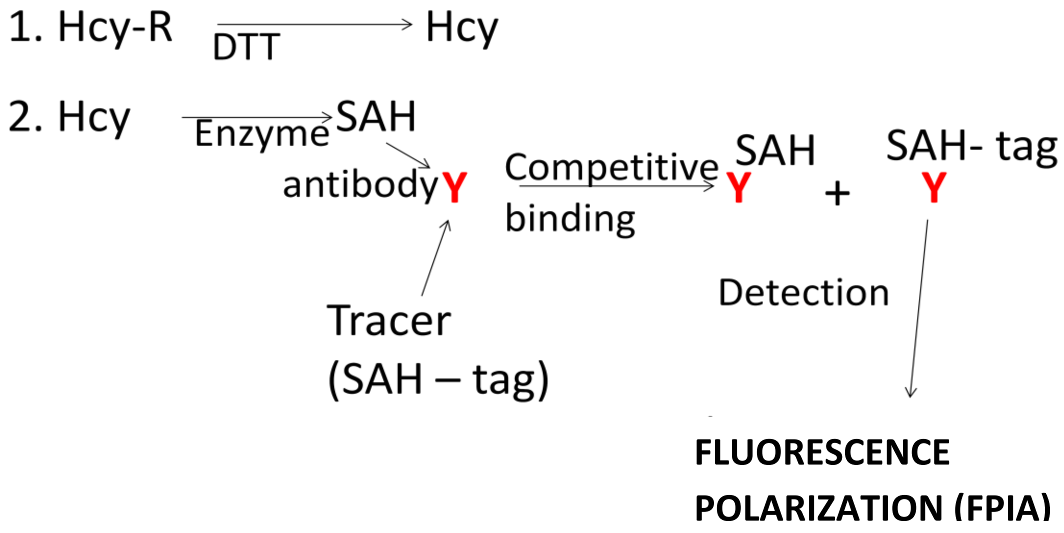
1. Chromatographic Method

The Chromatographic assay was developed in the early 1980's. It uses amino acid analyzer or High performance liquid chromatography (HPLC) and ion exchange column, which separate derivatized homocysteine molecules, depending on their retention times. This method is time consuming, can only analyze one sample at a time, and hence, is not suitable for a large number of samples. It is a labor–intensive method, and often requires skilled staff.

2. Immunoassay Method

The Immunoassay method, developed by Axis-Shield in the early 1990's, is based on the specific binding of an antibody with S-adenosyl homocysteine (SAH), a homocysteine enzyme conversion product. The antibody competes for SAH from the serum sample & from a tracer that is fluorescent chromophore tagged. After binding to the antibody, the detection of homocysteine is based on the fluorescent polarization of the tracer. The exact quantification of homocysteine is done by construction of standard curve with multiple known concentrations of calibrators.

Figure 4 showing mechanism of immunoassay for total homocysteine



3. **Enzyme cycling method**

This method of homocysteine estimation is fully automated. It uses minimal amount of plasma sample (< 20 microlitre), and the test can be performed in less than 10 minutes.

The HPLC method gives the best precision among all the three methods, but with increased cost, time and labor intensiveness. The enzyme cycling method is a cheaper and faster method with good precision. The immunoassay is quicker and cheaper than HPLC, but is less precise.(41)

Factors affecting Homocysteine estimation(37)

Homocysteine concentration increases with intake of protein rich diet. An increase of 10-15% is noted after 6-8 hours; hence, there is a diurnal variation, the Hcy levels being lowest in early hours of day and peaks in the evening.

Effect of posture at time of sample collection

There is a 10% decrease in the mean homocysteine concentration if the sample is collected in supine position as compared to sitting position, probably because of decrease in plasma albumin in supine position, as albumin binds with homocysteine.

Storage of sample

Till the separation of serum from the collected blood sample, there is a significant time- and temperature-dependent elevation in the homocysteine levels.

At room temperature, total homocysteine concentration increases at the rate of around 1µmol/L/hour. This increase is due to the continuous removal of Hcy from the red blood cells (RBCs) which can be prevented or minimised by immediate centrifugation to remove the RBCs from the sample, or by keeping the samples cooled on ice till centrifugation.

Hemolysis

Hemolysis has little effect on change in homocysteine concentration. Even for a small change in homocysteine concentration, hemolysis has to be extensive.(37)

Hyperhomocysteinemia and diabetic retinopathy

A number of studies have been done to determine the role of hyperhomocysteinemia in diabetic retinopathy. Some of these studies have found an association of hyperhomocysteinemia with diabetic retinopathy.

Brazionis et al. conducted a community-based, cross-sectional study on 168 subjects with T2DM to assess the relationship between Homocysteine and DR. They concluded that the mean concentration of Hcy was higher in the retinopathy group as compared to the no retinopathy group , which on multiple logistic regression was independent of other risk factors for DR. However, the difference in mean Hcy concentration between the two groups was only 2 µmol/L, and the homocysteine concentration in both groups was below the upper limit of normal.(11)

Malaguarnera et al. conducted a hospital-based study, and recruited T2DM patients, who were further divided into NPDR group (63 patients) and PDR group (62 patients). They also recruited fifty healthy subjects as controls, and 75 randomly selected patients. They found that the odds ratio for hyperhomocysteinemia was 4.24 and 1.16 in PDR and NPDR respectively. (13)

Goldstein M et al. conducted a hospital-based study in Israel to evaluate the prevalence of hyperhomocysteinemia (Hcy levels > 15 μmol/L) in diabetic patients with no retinopathy, with non proliferative and proliferative retinopathy. They included 179 patients with DM and 156 age-matched subjects without DM. They concluded that mean Hcy levels were significantly raised in the non proliferative and proliferative DR groups as compared to the control group. They also found a significantly higher prevalence of hyperhomocysteinemia in the non proliferative and proliferative DR groups as compared controls.(12)

The study done by Huang et al. included 257 patients with Type 2 diabetes mellitus, who were further classified on the basis of duration of diabetes and presence of retinopathy. They found that homocysteine concentration was significantly higher in patients with longer duration of diabetes (10 years as compared to 5 years). They concluded that homocysteine concentration was more in patients with DR as compared to without DR.(42)

Huijberts et al. found an association of hyperhomocysteinemia with microalbuminuria and DR in patients with both T1DM and T2DM.(43)

Fotiou et al. studied the association of serum Hcy levels with vitamin B12 and folic acid levels in Type 2 diabetic patients with retinopathy. They concluded hyperhomocysteinemia was an independent risk factor for DR, and that deficiencies of folic acid and vitamin B12 affect the risk of developing DR, through raised serum homocysteine concentration.(19)

Cho et al., studied the association between levels of homocysteine in plasma and incidence of retinopathy in Type 2 diabetics. They concluded that increased risk of retinopathy, was positively associated with hyperhomocysteinemia.(44)

The aim of the study by Bulum et al. was to determine if hyperhomocysteinemia was independently associated with diabetic retinopathy in normoalbuminuric patients with type1 DM (T1DM). They included 163 normoalbuminuric patients with eGFR > 60ml/1.73/m^2. They concluded that patients with retinopathy were older, with higher systolic blood pressure, triglyceride and Hcy levels. On logistic regression analysis, hyperhomocysteinemia was found to be independently associated with DR in normoalbuminuric patients with T1DM.(45)

The only published study on this subject from India was done by Satyanarayana et al. from Hyderabad, recruited 300 patients with T2DM. This included 200 patients with no DR and 100 patients with DR. They also recruited

100 normal (asymptomatic) subjects more than 50 years of age, without any cardiovascular and renal complications, as controls. They excluded subjects who were on vitamin supplementation. The mean plasma Hcy levels were significantly higher in the Type 2 diabetics with no retinopathy group compared to the normal controls group, which further increased ($p < 0.05$) in Type 2 diabetics with retinopathy group compared with the no retinopathy group.(23) They also found that the prevalence of hyperhomocysteinemia (>12 micromol/L), was significantly different ($p < 0.01$) among the three groups: 65% in diabetic retinopathy , 46% in no diabetic retinopathy and 11% in control groups. They also found that higher Hcy levels were associated with lower levels of folic acid and cynocobalamine. However, this study did not differentiate between proliferative and non-proliferative DR.(14)

There have been a few published studies, which failed to find an association between hyperhomocysteinemia and diabetic retinopathy.

The study by Agardh et al. assessed plasma homocysteine levels in patients with DM Type 1 with normoalbuminuria, microalbuminuria and proteinuria. This study found no relationship between plasma Hcy levels, either with different grades of DR, or with early stages of nephropathy.(16)

The study by Hultberg B et al. found elevated homocysteine levels in patients with PDR, as compared to patients with minimal or no DR, and control subjects. However, within the PDR group, increased homocysteine levels were confined to those patients with nephropathy. Patients with no or only minimal nephropathy had homocysteine levels that were not different from the control group. They concluded neither Type 1 diabetes nor diabetic retinopathy was associated with increased levels of homocysteine in blood. (15)

A systematic review and meta-analysis done in 2014, including 31 studies, to assess the relationship between serum homocysteine levels and diabetic retinopathy, concluded that hyperhomocysteinemia is associated with higher risk of DR. However, significant heterogeneity was observed in the different types of diabetics and different geographical locations of the studies. On subgroup analysis, this meta-analysis showed that hyperhomocysteinemia was associated with higher incidence rate of DR in patients with DM type 1 and in mixed (Type1+Type2) diabetes Patients with T2DM with hyperhomocysteinemia did not show higher risk of developing DR. In studies from Europe and Asia, hyperhomocysteinemia was found to be associated with diabetic retinopathy, especially PDR, but there was no significant difference observed in studies from Australia and USA. The authors of this meta-analysis concluded that hyperhomocysteinemia is a risk factor for DR, but due to heterogeneous results from different studies, further investigations are required to prove this association.(46)

MATERIALS AND METHODS

Design of study

This study was a hospital-based, case control study.

Study name

Role of homocysteine in proliferative diabetic retinopathy

Acronym: HARD (Homocysteine And Retinopathy in Diabetes)

Setting

This study was done in the Department of Ophthalmology, Christian Medical College(CMC), Vellore. CMC, Vellore is a tertiary care teaching centre in South India. The average number of patients seen per week in the outpatient clinics of the Department of Ophthalmology, CMC, Vellore, is 2000. The average number of patients admitted per week in the inpatient wards of the Department is 160.

Duration of study

The study was conducted over a period of eight months, from January 2015 to August 2015, after receiving the approval from Institutional Review Board (IRB Min No.9150, dated 12/11/14).

Patient selection

Patients with Type 2 DM, seen in the outpatient clinics of the Department of Ophthalmology, CMC, Vellore, from January 2015 to August 2015, were screened for eligibility for enrolment in the study. All patients underwent dilated fundus examination to diagnose and grade diabetic retinopathy, as part of their routine clinical evaluation.

Figure 5 showing slit lamp biomicroscopic fundus examination

Inclusion criteria

Patients with Type 2 diabetes mellitus (T2DM), seen in the outpatient clinics of the Department of Ophthalmology

Cases: Patients with Proliferative diabetic retinopathy (PDR)

Controls: Age & gender matched patients without diabetic retinopathy

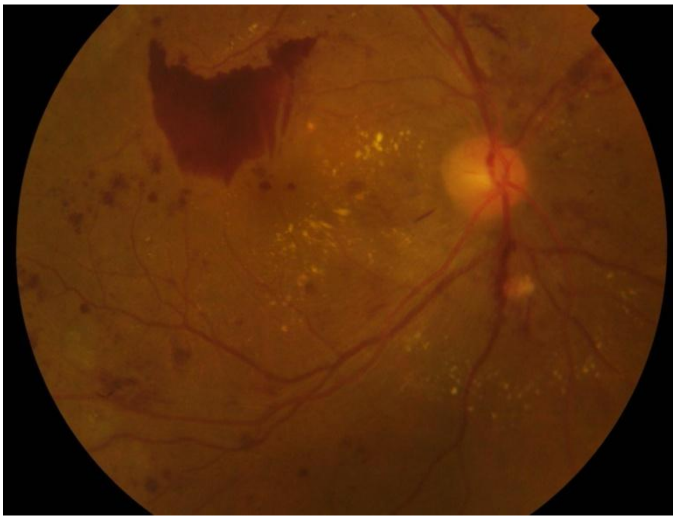


Figure 6 showing color fundus photograph of one of the cases in the study with proliferative diabetic retinopathy

Exclusion criteria

1. Age less than 40 years and more than 70 years
2. History of liver disease
3. Pregnant or post-partum women
4. Hazy ocular media in one or both eyes, precluding adequate visualization of the fundus for diagnosis and grading of diabetic retinopathy
5. Ocular diseases that may result in ambiguity in the diagnosis and grading of diabetic retinopathy such as retinal vessel occlusion, retinal vasculitis and retinal changes/ vitreous hemorrhage associated with ocular trauma

Methodology

Informed consent and enrolment in study

All patients were provided the information sheet regarding the study. Information sheets were available in English, Hindi and Tamil. For illiterate participants, the information sheet was read out in the language understood by them. Patients were then enrolled in the study after obtaining their informed consent.

A detailed questionnaire was administered to all the participants of the study. Measurement of blood pressure (BP) and estimation of Body mass index was done for all participants of the study. Ophthalmological investigations like Fundus fluorescein angiography and Optical coherence tomography, which were required for the routine management of diabetic retinopathy, were done as per standard clinical indications.

Fasting (AC) and two-hour postprandial blood sugar levels (PC), glycosylated hemoglobin (Hba1c), lipid profile (LDL levels), hemoglobin (Hb) and serum creatinine, if done within the past three months, were recorded. If these investigations were not available in the past three months, they were done as part of the standard of care for the management of diabetic patients.

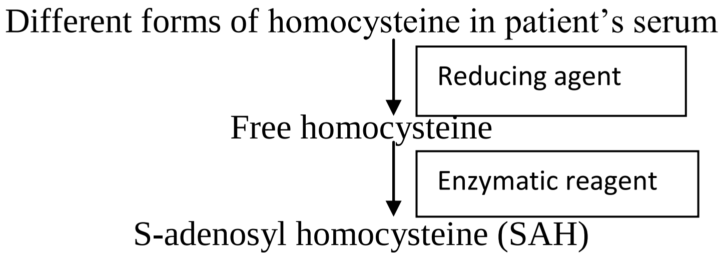
A fasting venous blood sample was collected from all participants for the estimation of serum homocysteine. Green tube containing heparinized lithium was used for sample collection, and serum homocysteine was analyzed by competitive immunoassay, using direct chemiluminescent technology.

Method used for Homocysteine estimation

ADIVA Centaur HYC competitive immunoassay

This method uses direct chemiluminescent technology for homocysteine estimation in the serum.

Figure 7 showing flow diagram of steps of homocysteine estimation



S-adenosyl homocysteine in the patient’s serum competes with S-adenosyl homocysteine that is covalently bound to paramagnetic particles in the solid phase, for acridium ester labelled anti –S-adenosyl hoocysteine in the lite reagent.

There exists an inverse relationship between the patient’s serum homocysteine level & the amount of Relative light units which are detected by the system.

Figure 8: Siemens Centaur XP used for the homocysteine assay at our Biochemistry laboratory

Diagrammatic algorithm of the study

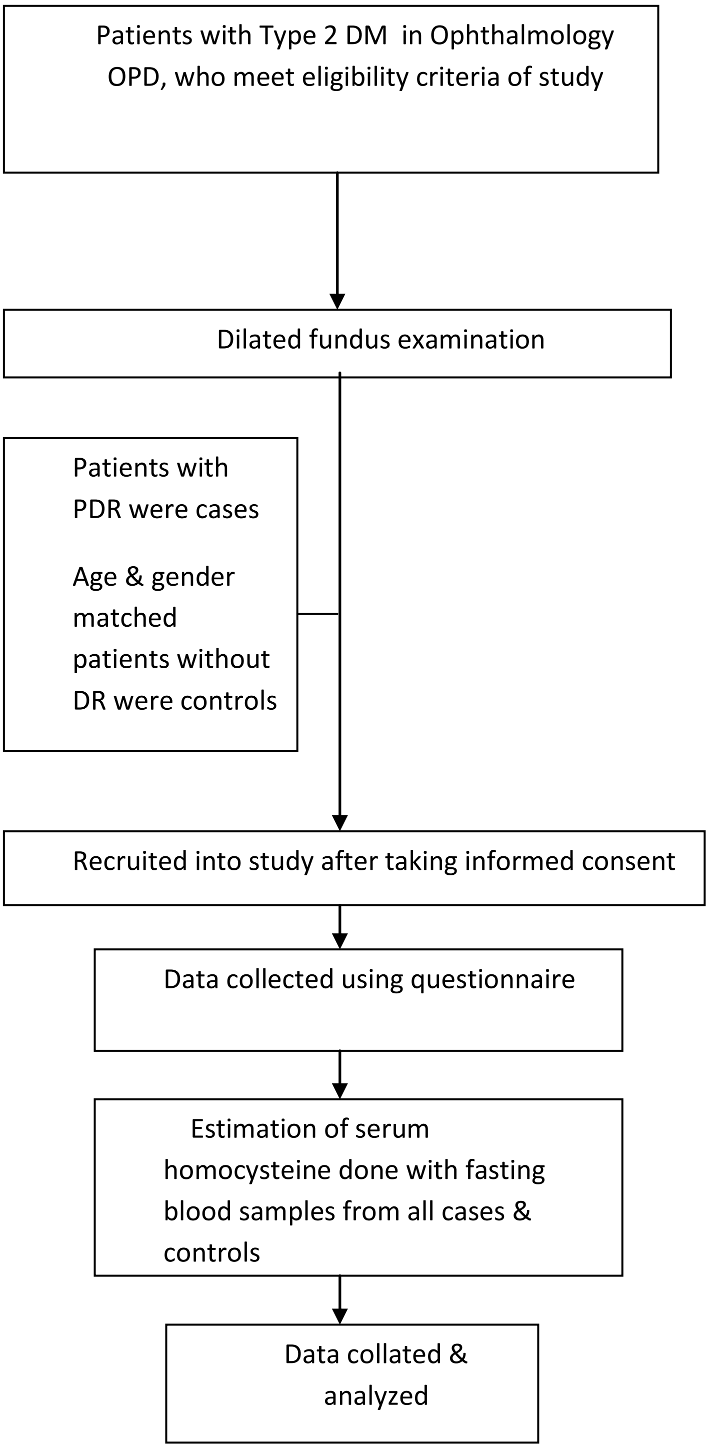


Figure 9 showing diagrammatic algorithm of the study

Sample size calculation

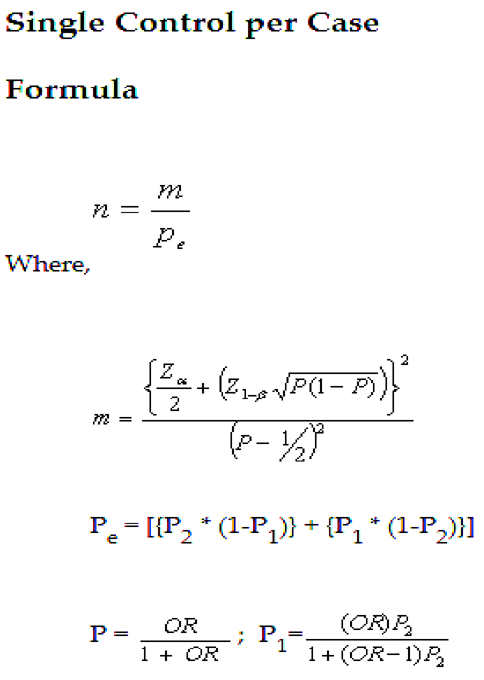
As hyperhomocysteinemia is still an emerging risk factor for diabetic retinopathy, different studies have taken different cut off values for hyperhomocysteinemia. Prevalence of hyperhomocysteinemia and odds ratios (range: 1.21 to 4.24) reported in various studies also show wide variation.(11,12,14,34) In this study, we tried to find if there is significant difference in the prevalence of hyperhomocysteinemia between the cases (with PDR) and the controls (without DR). We also wanted this difference to be clinically significant. Therefore, for the purpose of this study, we took the median serum homocysteine value of controls as the cut off for hyperhomocysteinemia. As a result, the prevalence of hyperhomocysteinemia in controls was 50%. Taking the odds ratio as 4 (for a clinically significant difference between the two groups), the sample size was calculated as given below:

Epidemiology Methods - Matched Case Control Studies - Single Control per Case

Proportion exposed in the control group	= .50
Anticipated Odds Ratio	= 4
Proportion exposed in the case group	= .80
Power (%)	= 80
Alpha Error (%)	= 5
Sided	= 2
Minimum no. of required discordant pairs, ' m '	= 19

No. of required pairs to detect ' m ' discordant pairs in the exposure group= 39

Alpha Error (%)	Power (%)	Sample Size (n)
1	70	50
	80	59
	90	72
5	70	31
	80	**39**
	90	50
10	70	24
	80	30
	90	39



Single Control per Case

Formula

$$n = \frac{m}{p_e}$$

Where,

$$m = \frac{\left\{\frac{Z_{\alpha}}{2} + \left(Z_{1-\beta}\sqrt{P(1-P)}\right)\right\}^2}{\left(P - \frac{1}{2}\right)^2}$$

$$P_e = [\{P_2 * (1-P_1)\} + \{P_1 * (1-P_2)\}]$$

$$P = \frac{OR}{1 + OR} \; ; \; P_1 = \frac{(OR)P_2}{1 + (OR-1)P_2}$$

P_1 : Calculated proportion in the case group

P_2 : Expected proportion exposed in the control group

OR : Estimated Odds ratio

n : Number of required pairs to detect m discordant pairs in the exposure group.

m : Minimum number of required discordant pairs.

α : Significance level

$1-\beta$: Power

With the proportion of exposed in the control group taken as 50% and anticipated odds ratio of 4, with a power of 80% and alpha at 5%, we calculated a sample size of 39 cases and 39 controls.

Data analysis

Data analysis was done using PASW Statistics 18 – SPSS software.

Statistical analysis

Descriptive statistics were reported using Mean ± SD for continuous variables, and n and (%) for categorical variables. Chi square test was used to assess the association of the categorical variables with the cases and controls. Two independent sample t test was used to compare the means between cases and controls, for continuous variables. The continuous variables like homocysteine levels, creatinine, eGFR, HbA1c, hemoglobin, LDL, BMI and age, were log transformed to achieve normality, and Pearson correlation coefficient was used to assess the strength of correlation between them.

Potential confounders/ suspected effect modifiers

Data regarding confounding factors/ suspected effect modifiers were obtained by history/ clinical examination/ laboratory investigations. The following were considered as potential confounders/ suspected effect modifiers:

Age, gender, duration of diabetes, glycemic control, hypertension, anemia, hyperlipidemia, nephropathy, smoking, alcoholism, obesity, malabsorption syndromes, use of medication like oral contraceptive pills, diuretics, metformin, fibrates, folate, vitamin B12, vitamin B6, multivitamin supplementation, nicotinic acid, antiepileptics, levodopa, sulfasalazine, trimethoprim, pyrimethamine, methotrexate, proton pump inhibitors and histamine 2 receptor antagonists.

Age and gender were matched between cases and controls. Other factors like duration of diabetes, glycemic control, hypertension, anemia, hyperlipidemia, nephropathy, smoking, alcoholism, obesity, malabsorption syndromes and use of medication were documented and analyzed.

Management of bias

The laboratory personnel who processed and analyzed the blood samples were masked towards the group to which the patient belonged (whether case or control).

Diagnostic criteria/ definitions

1. Criteria for diagnosis of Type 2 diabetes

- Onset of diabetes at age >30 years
- Family history of diabetes present
- No symptoms of ketosis at initial diagnosis
- Initially controlled on oral hypoglycemic agents (even if on insulin at present)
- Overweight (Increased BMI)

2. Proliferative diabetic retinopathy

The presence and level of DR were assessed by dilated fundus examination using slit lamp binocular indirect ophthalmoscopy. DR was classified based on Modified Airlie House Classification (Early Treatment Diabetic Retinopathy Study.(2)

PDR

Composed of NVD or NVE, pre-retinal or vitreous hemorrhage , fibrous tissue proliferation

Early PDR (Level E)

New vessels

Definition not met for F

High Risk PDR (Level F)

NVD (1/3-1/2 disc area)

NVD and Vitreous or pre-retinal hemorrhage

NVE ≥ ½ disc area and Vitreous or pre-retinal hemorrhage

3. Hyperhomocysteinemia

The normal homocysteine concentrations range from 5 - 15 micromol /L.(38,47)

Hyperhomocysteinemia classification:

Moderate ranges from 15 - 30 micromol/L

Intermediate ranges from30 - 100 micromol/L

Severe is defined as more than 100 micromol/L

However, as hyperhomocysteinemia is still an emerging risk factor for diabetic retinopathy, different studies have taken different cut off values for hyperhomocysteinemia. Prevalence of hyperhomocysteinemia and odds ratios reported in various studies has also shown wide variation. Therefore, for the purpose of this study, the median serum homocysteine value of controls (≥16.19 µmol/L) was taken as the cut off for hyperhomocysteinemia. We also performed analysis with serum homocysteine levels of ≥10 micromol/L, ≥12 micromol/L and ≥15 micromol/L as cut off values for hyperhomocysteinemia.

4. Glycemic control

HbA1C level for good glycemic control <7%.(48)

5. Hypertension

Known hypertensive on treatment, or if a recording of elevated blood pressure as below:

- Systolic ≥ 140 mmHg or diastolic ≥ 90 mmHg

The diagnosis was based on the average of ≥ 2 correctly measured readings each at 2 or more visits following an initial screen.(49)

6. Anemia

Previously diagnosed anemia on treatment, or

Hemoglobin in men and women <13 and <12 g/dL, respectively (50)

7. Lipid profile

Known case of hyperlipidemia on treatment, or if

LDL cholesterol ≥100 mg/dL (48)

8. Kidney disease

Chronic kidney disease is defined as the presence of kidney damage or decreased kidney function (defined as estimated glomerular filtration rate [eGFR] <60 mL/min per 1.73 m^2) for three or more months, irrespective of the cause.(51)

For the purpose of this study, kidney dysfunction (likely to cause hyperhomocysteinemia due to decreased renal clearance) was defined as eGFR less than 60 mL/min per 1.73 m^2) irrespective of the cause.

9. Smoking

All participants were categorized into four groups:

- Group 1 (Nil smoking) – participants who did not smoke at all
- Group 2 (Regular smoking) – participants who smoke more than once a week
- Group 3 (Occasional smoking) – participants who smoke less than once a week
- Group 4 (Quit smoking) – participants who stopped smoking at least six months earlier

For the purpose of this study, smoking was considered as positive in all those falling under group 2.

10. Alcoholism

All participants were divided into the following four groups:

- Group 1 (Nil alcohol consumption) – participants who did not consume alcohol at all
- Group 2 (Regular alcohol consumption) – participants who consume alcohol more than once a week

- Group 3 (Occasional alcohol consumption) – participants who consume alcohol more than once a week
- Group 4 (Quit alcohol consumption) – participants who stopped alcohol consumption at least six months earlier

For the purpose of this study, alcoholism was considered as positive in all those falling under group 2

11. Body mass index (BMI)

BMI is measured as body weight (in kg) ÷ height (in meters) squared (52)

Based on the calculated BMI, the participants were categorised as follows:

1-Underweight: BMI less than 18.5 kg/m^2

2-Normal weight: BMI ≥ 18.5 - 24.9 kg/m^2

3-Overweight: BMI ≥ 25.0 - 29.9 kg/m^2

4-Obesity: BMI more than or equal to 30 kg/m^2

RESULTS

Thirty nine patients with Proliferative diabetic retinopathy (PDR), who fulfilled the eligibility criteria, were recruited into the study, as cases after taking informed consent. Thirty nine age and gender-matched patients without diabetic retinopathy (No DR) were recruited into the study as controls after taking informed consent.

Demographic Profile

Age -The mean (± SD) age in the case and control groups was 55.3 ± 5.4 years and 54.8 ± 6.1 years respectively (p = 0.7).

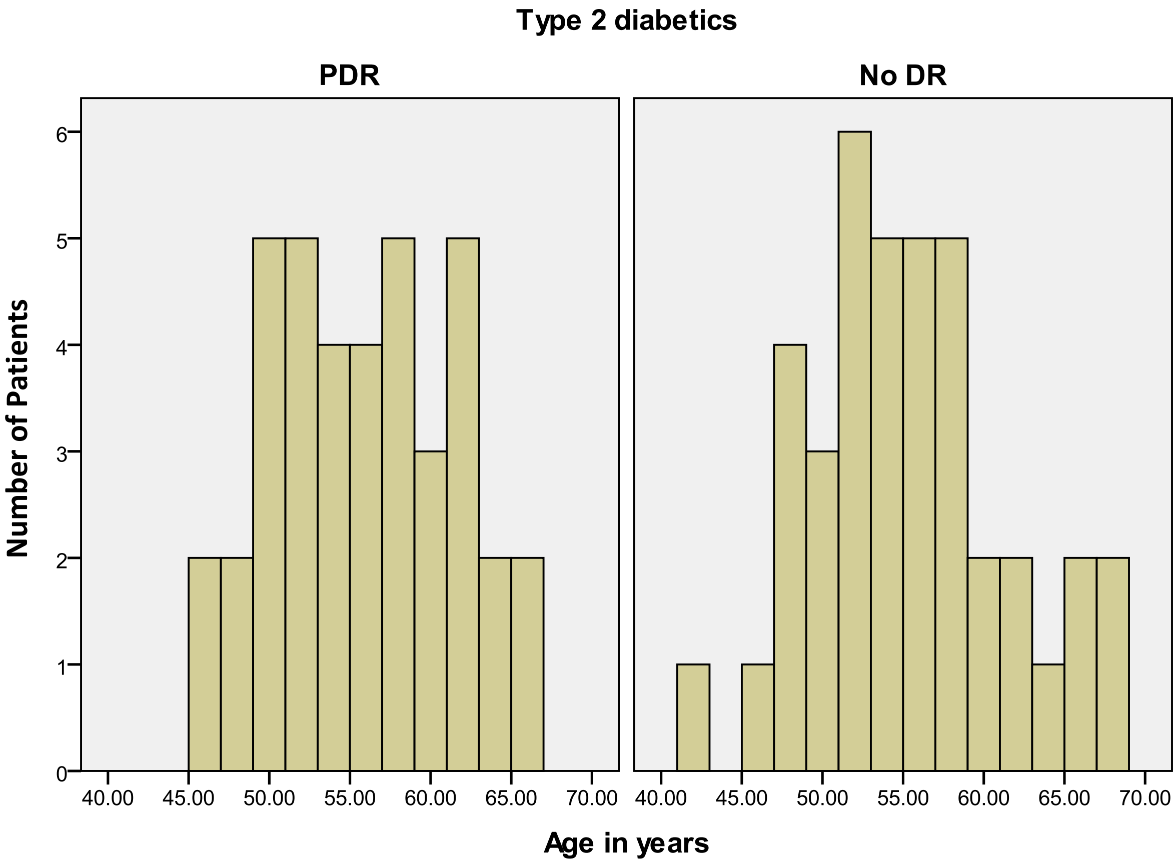


Figure10 showing distribution of age in the two groups

Gender

There were 33 men and 6 women in each group. The cases and controls were matched for age and gender.

Place of residence

Among the cases, 27 (69.2%) were residents of Tamil Nadu, while 26 (66.7%) controls were residents of Tamil Nadu (p = 0.80).

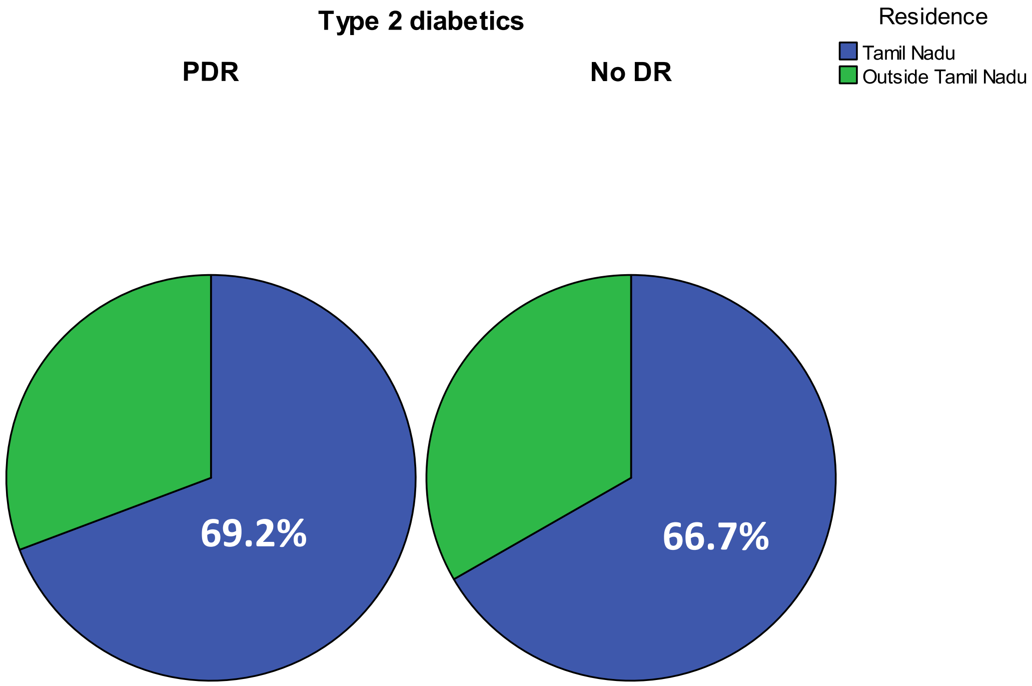


Figure 11 showing demographic location of the patients in the two groups

Dietary habits

There was no statistically significant difference between the two groups with respect to their diet (vegetarian diet: 5 cases (12.8%) vs. 7 controls (17.9%); p = 0.53).

Table 1: Dietary habits of the two groups

Diet	PDR n(%)	No DR n(%)	p-value
Vegetarian	5(12.8)	7(17.9)	0.53
Non-vegetarian	34(87.2)	32(82.1)	

All baseline demographic variables were comparable between the two groups.

Table 2 gives the summary of the demographic profile of the two groups.

Table 2: Demographic profile of the two groups

Variable	PDR (n=39) Mean±SD or n (%)	No DR (n=39) Mean±SD or n (%)	p-value
Age (yrs)	55.3±5.4	54.8±6.1	0.70
Male	33(84.6)	33(84.6)	1.0
Female	6(15.4)	6(15.4)	
Residence			
In Tamil Nadu	27(69.2)	26(66.7)	0.80
Outside Tamil Nadu	12(30.8)	13(33.3)	
Vegetarian diet	5(12.8)	7(17.9)	0.53
Non-vegetarian diet	34(87.2)	32(82.1)	

Risk factors

Duration of diabetes

The duration of diabetes (>15 years) was significantly higher in the proliferative retinopathy group (cases) as compared to the no retinopathy group (controls) (35.9% of cases vs. 2.6% of controls).

Table 3: Duration of diabetes in the two groups

Duration of diabetes (in years)	PDR n(%)	No DR n(%)	p-value
< 5	6(15.38)	18(46.15)	**<0.01**
5-10	12(30.77)	10(25.64)	
>10-15	7(17.95)	10(25.64)	
> 15	14(35.90)	1(2.56)	

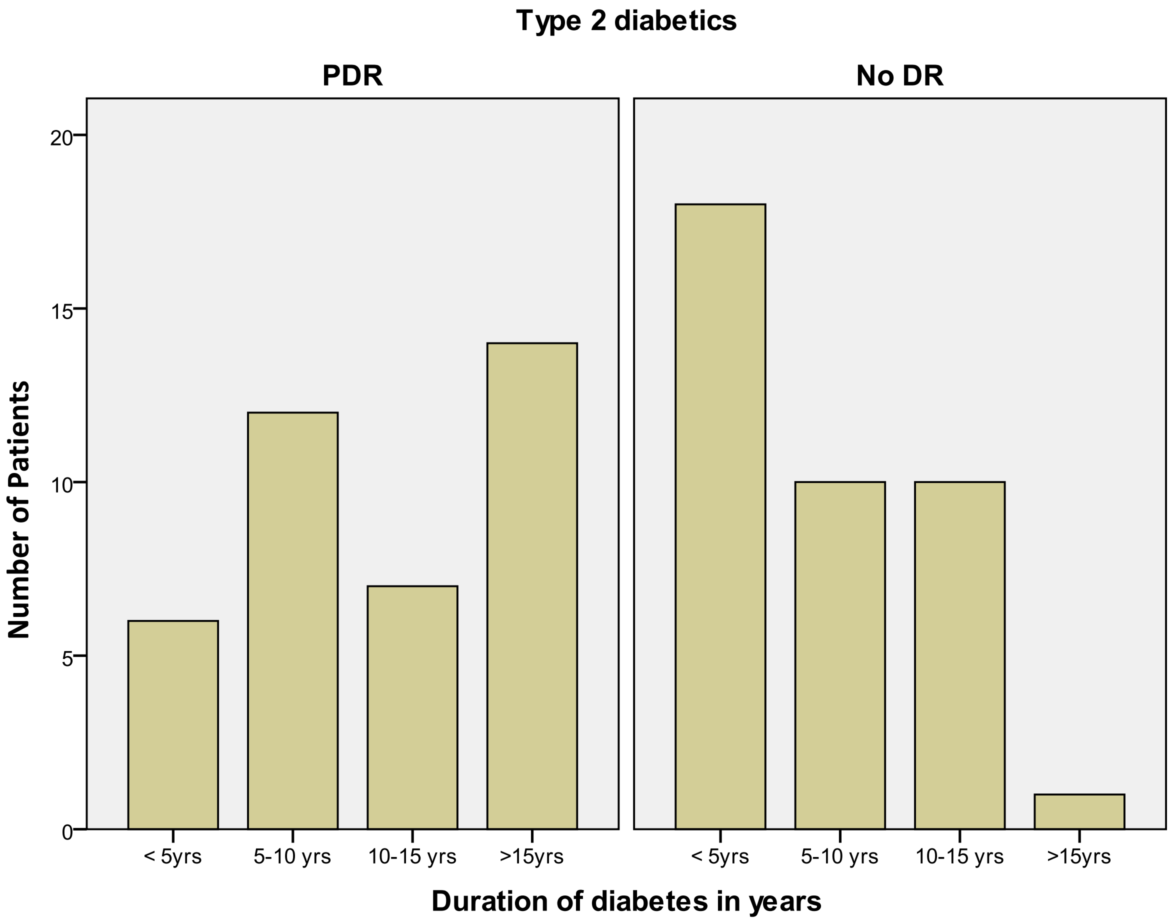


Figure 12 showing duration of diabetes in the two groups

Glycemic control

The mean (± SD) of HbA1c was higher among cases as compared to controls (8.6 ± 2.4% vs. 7.7 ± 1.3%; p = 0.05) with borderline significance, although there was no significant difference between the two groups with respect to glycemic control. Poor glycemic control (defined as HbA1c > 7%) was present in 64.1% of cases vs. 71.8% of controls; p = 0.47).

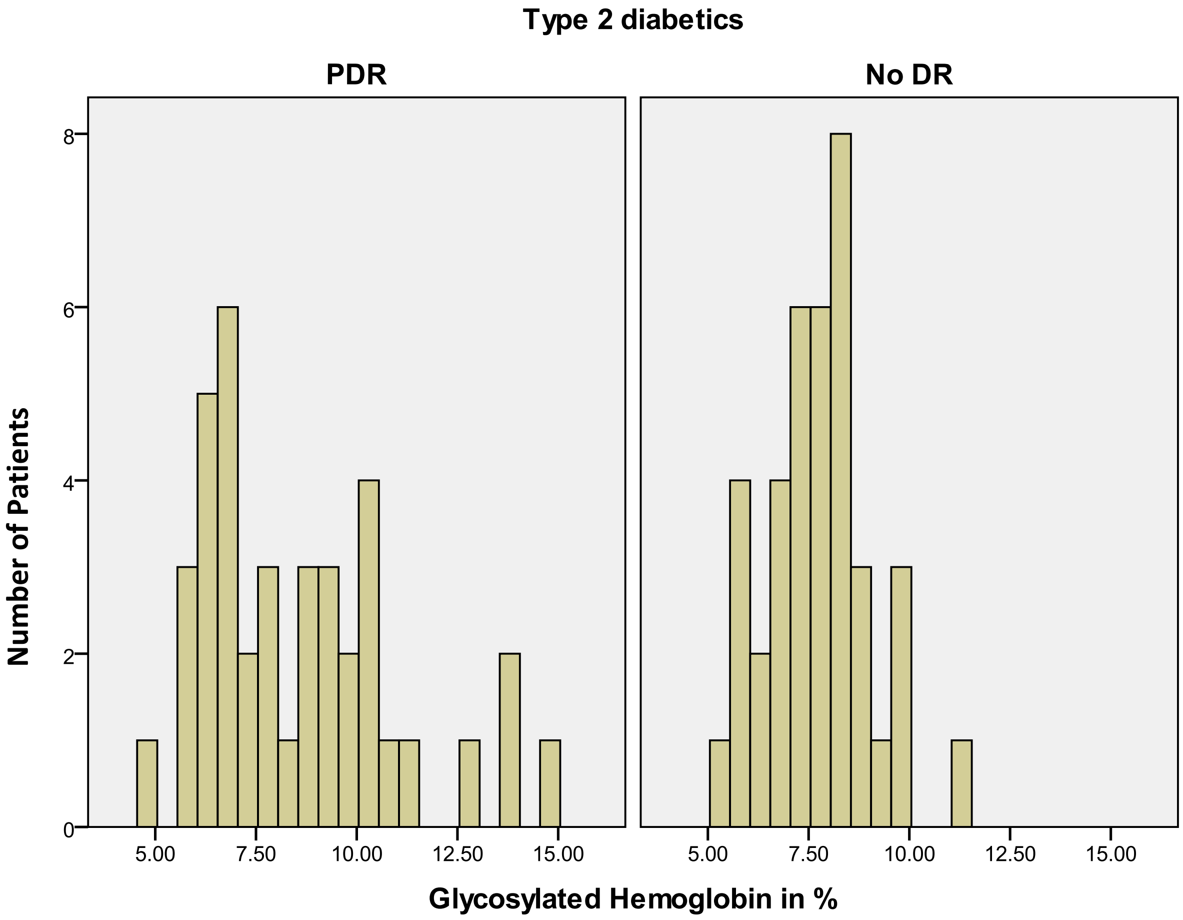


Figure13 showing distribution of glycosylated hemoglobin in the two groups

Table 4: Glycemic control in two groups

Glycemic control	PDR n(%)	No DR n(%)	p-value
Poor	25(64.1)	28(71.8)	0.47
Good	14(35.9)	11(28.2)	

Hypertension

The mean (± SD) systolic BP was significantly elevated among the cases as compared to controls (137.4 ± 18.8 mmHg vs. 126.4 mmHg ± 16.1 mmHg; p = 0.01), though there was no significant difference in the mean (± SD) diastolic BP between the two groups (83 ± 10 mmHg vs. 79.7 ± 8.9 mmHg; p = 0.12). The prevalence of hypertension (defined as systolic BP > 140 mmHg or diastolic BP > 90 mmHg, or known hypertensive on treatment) was significantly higher in cases as compared to the controls (84.6% vs. 51.3%; p < 0.01).

Table 5: Comparison of blood pressure in the two groups

	PDR Mean±SD or n(%) n=39	No DR Mean±SD or n(%) n=39	p-value
Systolic BP (mmHg)	137.4±18.8	126.4±16.1	**0.01**
Diastolic BP (mmHg)	83.0±10.0	79.7±8.9	0.12
Hypertension	33(84.6)	20(51.3)	**<0.01**

Anemia

The mean (± SD) of hemoglobin levels was significantly lower in cases as compared to controls (11.62 ± 1.96 g/dl vs. 13.52 ± 1.62 g/dl; $p < 0.001$). Similarly, the prevalence of anemia (defined as Hb levels < 13 g/dl in males and < 12 g/dl in females) was significantly higher in cases as compared to controls (74.4 % vs. 28.2%; $p < 0.001$).

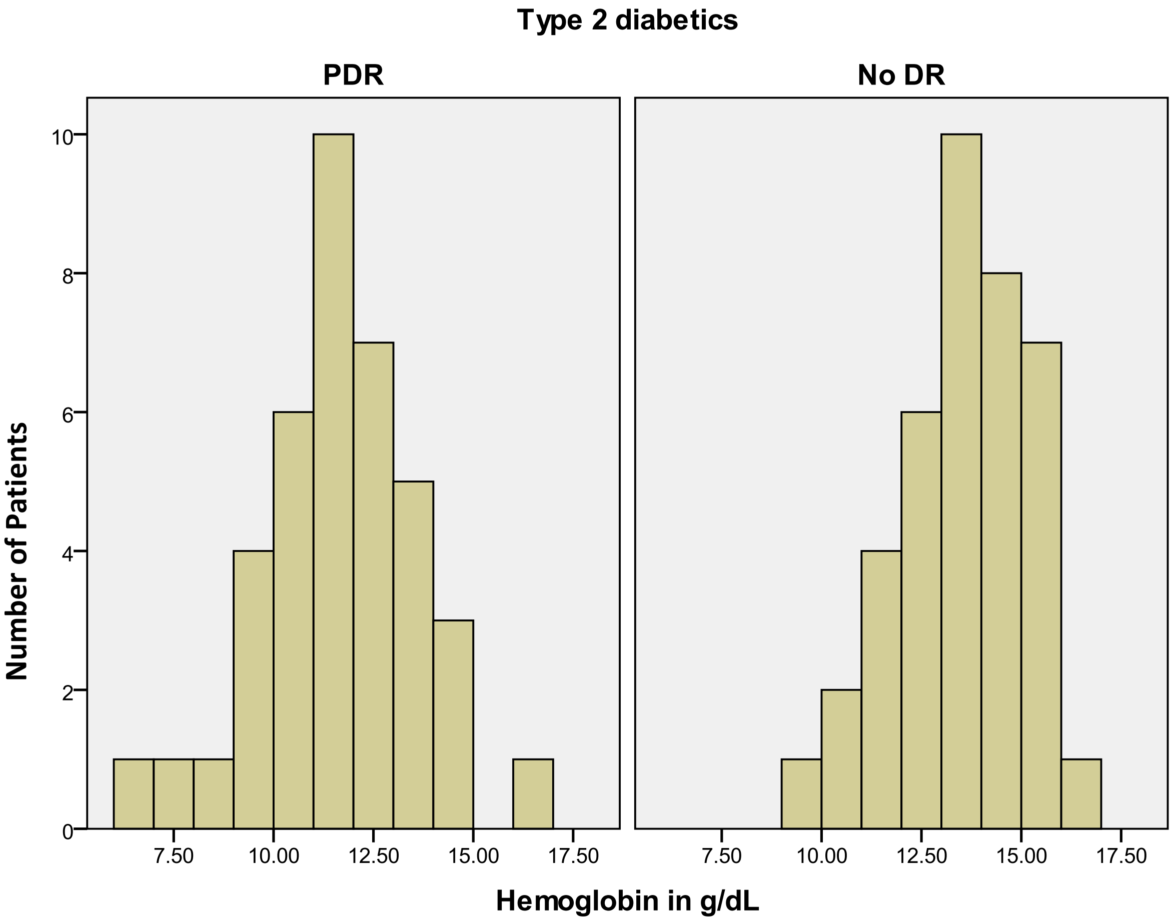


Figure 14 showing the distribution of hemoglobin values in the two groups

Hyperlipidemia

The mean (± SD) of serum LDL levels was higher in cases as compared to controls, with borderline significance (112.9 ± 40.0 mg/dL vs. 96.6 ± 32.7 mg/dL; p = 0.05), though prevalence of hyperlipidemia (defined as LDL levels > 100 mg/dL or known case of hyperlipidemia receiving treatment) was not significantly different between the two groups (82.1% vs. 66.7%; p = 0.12).

Table 6: Hyperlipidemia in the two groups

Hyperlipidemia	PDR	No DR	p-value
Hyperlipidemia	26(66.67)	32(82.1)	0.12
No Hyperlipidemia	13(33.33)	7(17.9)	

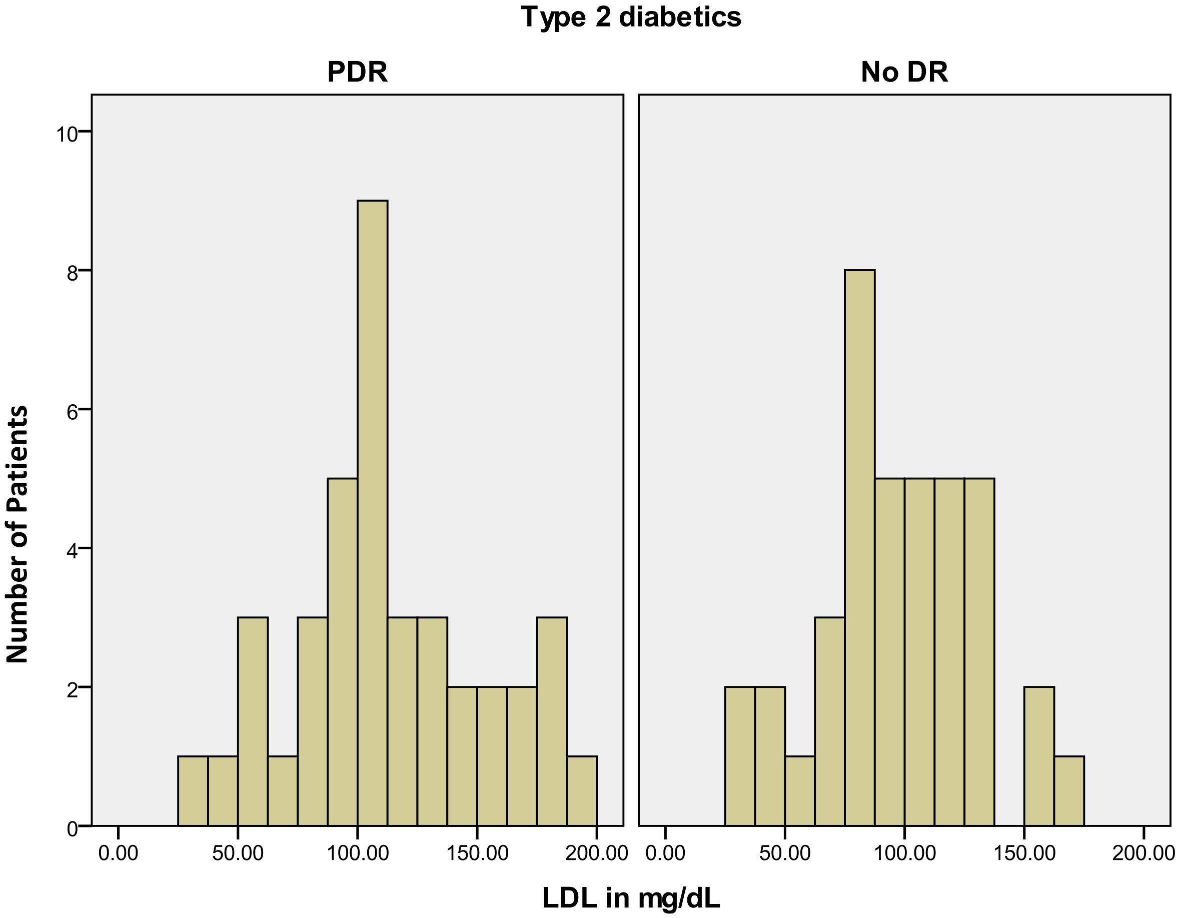


Figure 14 showing LDL levels in the two groups

Renal dysfunction

There was significant renal dysfunction in cases, as evidenced by significantly raised mean (± SD) creatinine levels (1.75 ± 1.33 mg/dL vs. 0.89 ± 0.27 mg/dL; $p < 0.001$), and significantly declining mean (± SD) eGFR (62.84 ± 33.01 ml/min/1.73 m^2 in cases vs. 92.43 ± 23.59 ml/min/1.73 m^2 in controls; $p < 0.001$). The prevalence of renal dysfunction (defined as eGFR < 60 ml/min/1.73 m^2) was also significantly higher among the cases as compared to controls (41% vs. 7.7 %; $p < 0.001$). (Table 7)

Table 7: Renal profile of the two groups

Variables	**PDR** **n=39** **mean±SD or n(%)**	**No DR** **n=39** **mean±SD or n(%)**	**p-value**
Creatinine (mg/dl)	1.75±1.33	0.89±0.27	**<0.001**
eGFR (ml/min/1.73m^2)	62.84±33.01	92.43±23.59	**<0.001**
Renal dysfunction	17(43.6)	3(7.7)	**<0.001**

Smoking

Prevalence of smoking in cases and controls was 7.7% and 2.6 % respectively, and there was no statistically significant difference between the two groups.

Table 8: Prevalence of smoking in the two groups

	PDR	No DR	p-value
Smoking	3(7.7)	1(2.6)	0.6
No smoking	36(92.3)	38(97.4)	

Alcoholism

There were no alcoholics among the cases, while there was one alcoholic among the controls.

Table 9: Prevalence of alcoholism in the two groups

	PDR	No DR	p-value
Alcoholism	0	1(2.6)	1.0
No Alcoholism	39 (100)	38(97.4)	

Obesity

The mean (± SD) of Body mass Index (BMI) was similar in the two groups (26.3 ± 4.1 kg/m^2 in cases vs. 27.0 ± 4.2 kg/m^2 in controls; p = 0.5).

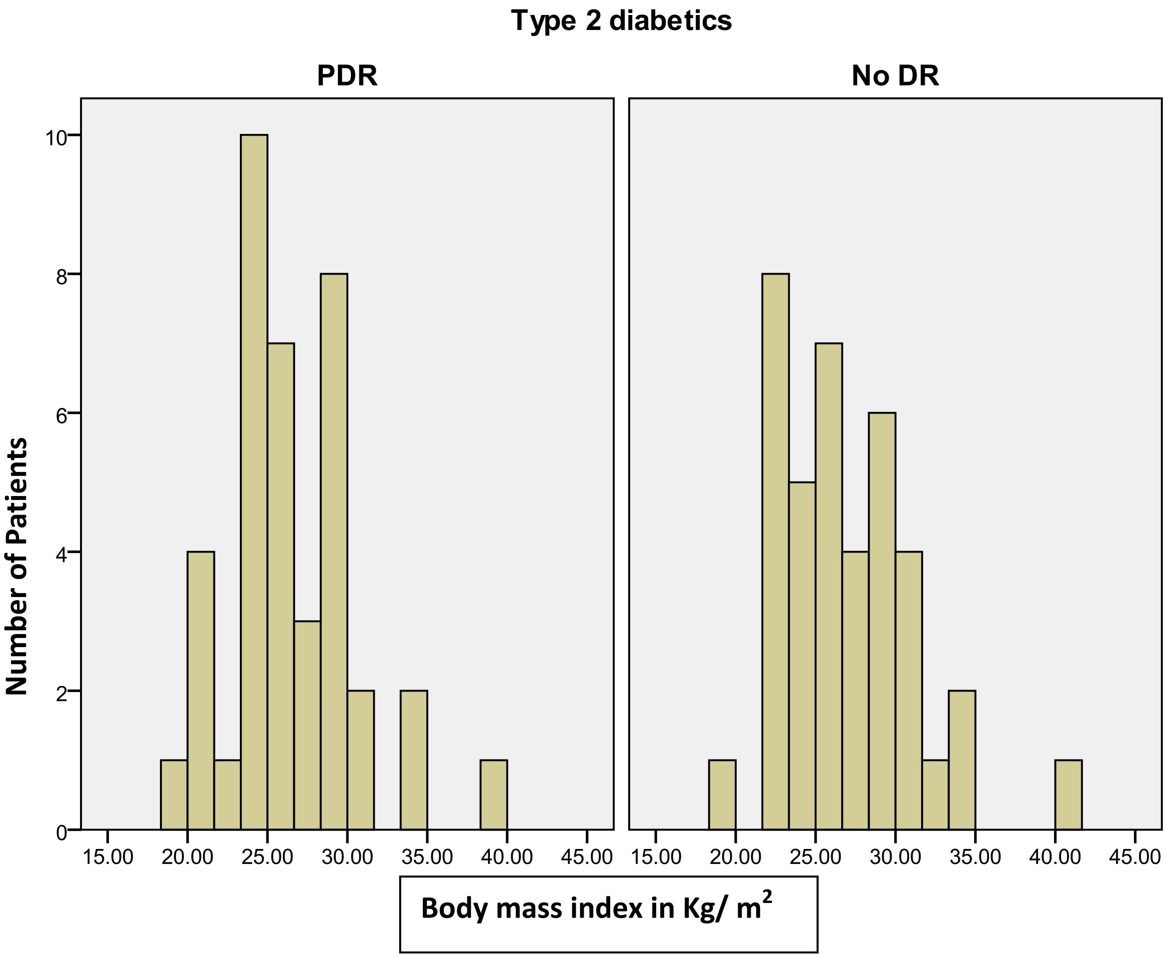


Figure 15 showing BMI in the two groups

Malabsorption syndromes

Neither group had any patient with malabsorption syndromes.

Table 10 summarizes the risk factors in the two groups.

Table 10: Comparison of the two groups with respect to the risk factors

Risk factors	PDR n=39 mean ± SD or n(%)	No DR n=39 mean ± SD or n(%)	p-value
Duration of diabetes			
<5 yrs	6(15.4)	18(46.2)	
5-10 yrs	12(30.8)	10(25.6)	**<0.001**
>10-15 yrs	7(17.9)	10(25.6)	
>15yrs	14(35.9)	1(2.6)	
Poor glycemic control	25(64.1)	28(71.8)	0.4
Good glycemic control	14(35.9)	11(28.9)	
Hypertension	33(84.6)	20(51.3)	**<0.01**
No hypertension	6(15.4)	19(48.7)	
Anemia	29(74.4)	11(28.2)	**<0.001**
No anemia	10(25.6)	28(71.8)	
Hyperlipidemia	26(66.7)	32(82.1)	0.12
No hyperlipidemia	13(33.3)	7(17.9)	
Renal dysfunction	17(43.6)	3(7.7)	**<0.001**
No renal dysfunction	22(56.4)	36(92.3)	
Smoking	3(7.7)	1(2.6)	0.6
No smoking	36(92.3)	38(97.4)	
Alcoholism	0(0)	1(2.6)	1
No alcoholism	39(100)	38(97.4)	
BMI	26.3±4.1	27.0±4.2	0.47

Drug history

There was a statistically significant difference between the two groups with the respect to the use of drugs such as metformin, diuretics and folate supplementation, with the use of metformin being more common in controls, and the use of diuretics and folate supplementation being more common in cases. Though the use of drugs like fibrates, multivitamins, vitamin B12, vitamin B6, nicotinamide and proton pump inhibitors was more in the cases as compared to the controls, the difference was not statistically significant. There was also no significant difference between the two groups with respect to the intake of drugs like antiepileptics and H2 blockers. There was no history of intake of other drugs like levodopa, sulfalsalazine, trimithoprim, pyrimethamine, methotrexate and oral contraceptive pills, in either of the two groups. Table 11 summarizes the details of drug intake in the two groups.

Table 11: Comparison of the two groups with respect to drug intake

Variables	PDR n=39	%	No DR n=39	%	p-value
Metformin	18	46.2	36	92.3	**<0.001**
Diuretics	12	30.8	3	7.7	**0.01**
Folate	10	25.6	0	0	**0.001**
Fibrates	2	5.1	0	0	0.49
Multivitamins	7	17.9	3	7.7	0.18
Cyanocobalamin	9	23.1	4	10.3	0.13
Vitamin B6	6	15.4	3	7.7	0.48
Nicotinamide	7	17.9	3	7.7	0.18
Proton pump inhibitor	7	17.9	2	5.1	0.15
Antiepileptics	0	0	1	2.6	1
H2 Blocker	0	0	1	2.6%	1
Levodopa	0	0	0	0	
Sulfasalazine	0	0	0	0	
Trimithoprim	0	0	0	0	
Pyrimethamine	0	0	0	0	
Methotrexate	0	0	0	0	
Oral Contraceptive pills	0	0	0	0	

Hyperhomocysteinemia

Although the mean (± SD) levels of serum homocysteine was higher among the cases as compared to the controls, the difference was not statistically significant (17.98 ± 6.26 µmol/L in cases vs. 17.71 ± 8.17 µmol/L in controls; p = 0.87).

Table 12: Levels of homocysteine in the two groups

Homocysteine	n	Mean	S.D.	Min.	Median	Max.	p-value
PDR	39	17.98	6.26	7.04	16.8	33.45	0.87
No DR	39	17.71	8.17	6.95	16.19	41.48	

Homocysteine levels in micromol/L

There was no statistically significant difference in the prevalence of hyperhomocysteinemia between cases and controls, when hyperhomocysteinemia was defined as serum homocysteine level ≥16.19 µmol/L, which was the median value of serum homocysteine in controls (59% vs. 48.7%; p = 0.36); neither was there any statistically significant difference between cases and controls when hyperhomocysteinemia was defined as serum homocysteine levels ≥ 10 µmol/l, ≥ 12 µmol/l or ≥ 15 µmol/l. (Table 13)

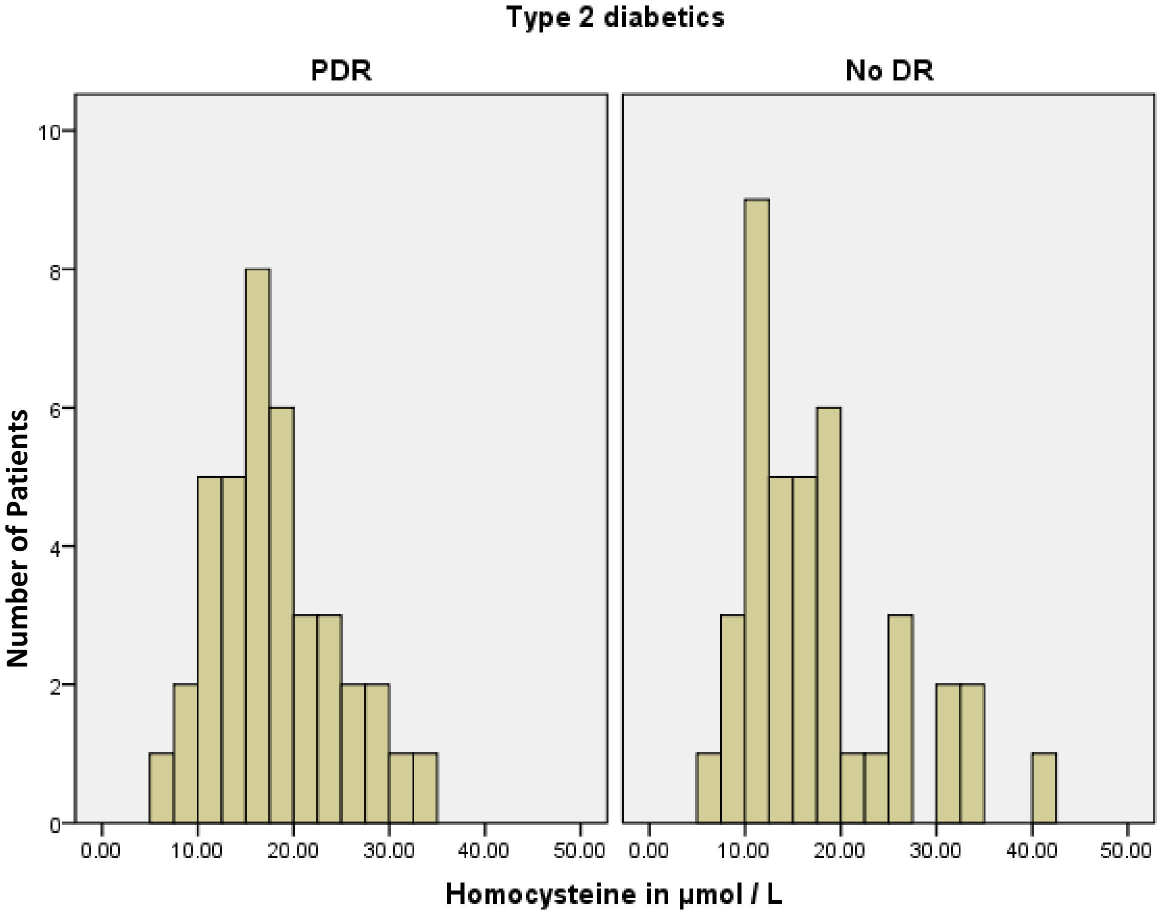


Figure 16 showing serum homocysteine level in the two groups

Table13: Serum homocysteine and retinopathy

Variables	**PDR n=39 n(%)**	**No DR n=39 n(%)**	**p-value**
Homocysteine ≥ 16.19 micrmol/L	23(59.0)	19(48.7)	0.36
Homocysteine ≥ 10 micromol/L	36(92.3)	35(89.7)	1.0
Homocysteine ≥ 12 micromol/L	33(84.6)	30(76.9)	0.39
Homocysteine ≥ 15 micromol/L	25(64.1)	21(53)	0.36

Correlation of serum homocysteine levels with other risk factors

There was no significant correlation (r = 0.01; p = 0.97) between serum homocysteine and creatinine levels in the cases (PDR), though there was a statistically significant positive correlation (r = 0.32; p = 0.046) between the serum homocysteine and creatinine levels in the controls.

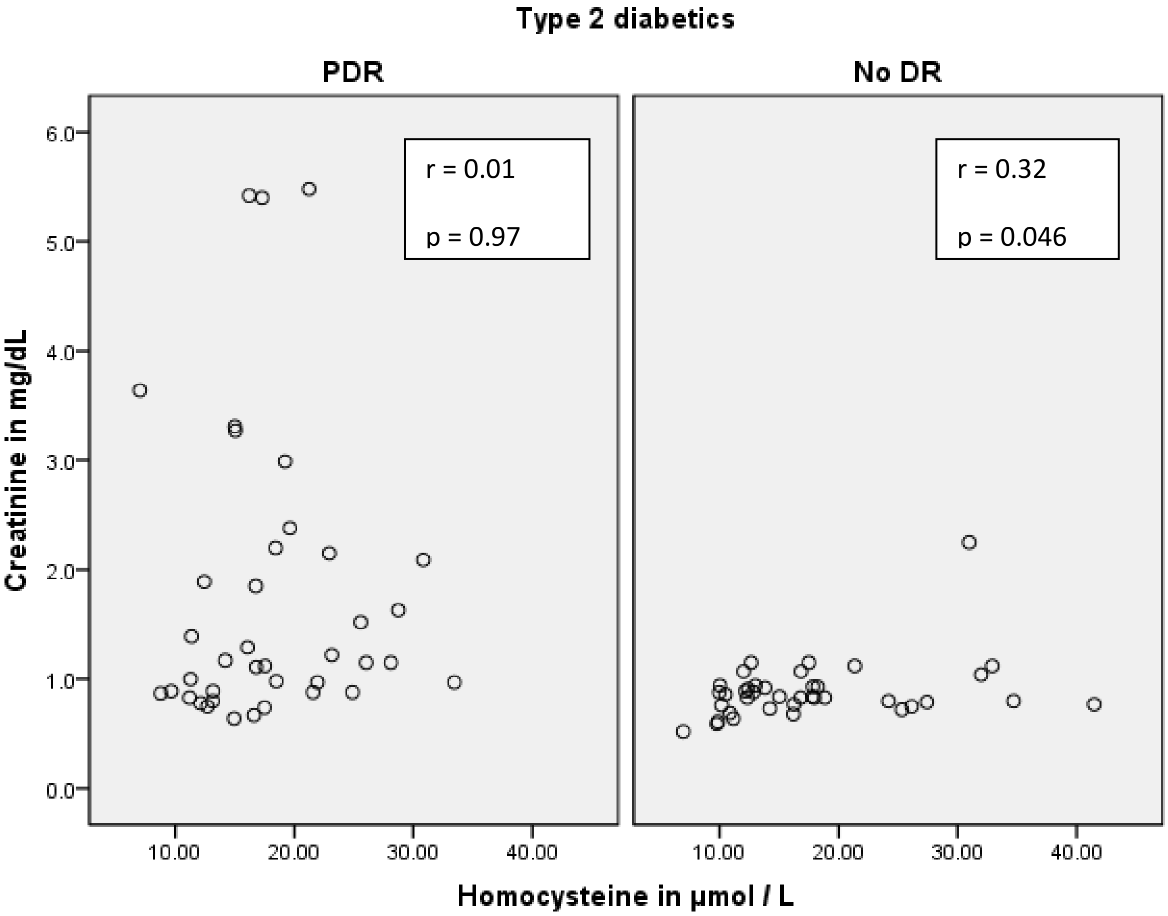


Figure 17 showing correlation between serum homocysteine and creatinine levels in the two groups

Among other variables like eGFR, HbA1c, hemoglobin, LDL, BMI and age, we were not able to demonstrate any statistically significant correlation with serum homocysteine, in either cases or controls.

Table 14: Correlation coefficient of serum homocysteine levels with other risk factors in the two groups

Risk factor	PDR Homocysteine level (Correlation coefficient r)	p-value	No DR Homocysteine level (Correlation coefficient r)	p-value
Creatinine	0.01	0.97	0.32	0.046
eGFR	-0.22	0.18	-0.27	0.1
HbA1c	0.04	0.80	-0.23	0.16
Hemoglobin	-0.19	0.24	-0.01	0.93
LDL	0.05	0.78	-0.21	0.20
BMI	0.03	0.85	-0.26	0.12
Age	0.15	0.35	0.21	0.19

DISCUSSION

The global prevalence of diabetic retinopathy (DR) among diabetics is 34.6%.(53) With the increasing prevalence of diabetes in India, its ocular complications are also on the rise, The prevalence of diabetic retinopathy, as estimated in the CURES Eye study, was 17.6% among diabetic patients.(5) The prevalence of DR was reported to be 22.4% in southern India as per the Andhra Pradesh Eye Disease Study (APEDS).(54)

DR is one of the leading causes of blindness among the adult population suffering from diabetes.(55) There are several risk factors that are well known to cause progression of retinopathy. Some factors like age, duration of diabetes and genetic predisposing factors are non-modifiable. On the other hand, risk factors like hyperglycemia, hypertension, anemia, hyperlipidemia, obesity and nephropathy are modifiable to varying extents. In the last decade, elevated homocysteine level in the blood has emerged as a novel risk factor for the progression and development of DR.(55) However, there are a few studies that have failed to show an association of hyperhomocysteinemia with progression of diabetic retinopathy.(15,16)

Therefore, there has been no definite evidence so far, to prove or disprove this association. Since supplementation of vitamin B12 and folate has been shown to reduce homocysteine levels in serum to varying extents in different studies, hyperhomocysteinemia may be a modifiable risk factor.(56,57) Hence, understanding and characterizing the role of hyperhomocysteinemia in the pathogenesis of DR may help in identifying a novel target to combat this potentially blinding disease.

Defining hyperhomocysteinemia

Although hyperhomocysteinemia is being studied as a possible risk factor for ocular microvascular complications, there are disparities between various studies conducted on this subject. The cut-off for defining hyperhomocysteinemia is arbitrary, and has differed substantially among different studies. The global disparity in defining hyperhomocysteinemia may be due to different genetic constitutions, life styles, environmental, nutritional and dietary factors. The cut-off values defined in various studies range from 12 µmol/L - 16 µmol/L.(19,58)

Demographic profile

The serum homocysteine levels do depend upon the age profile of patients, as described by Moat et al.(59) We, therefore, chose age-matched controls for each of our cases with PDR. The mean age of patients with DR and no DR was 55.8± 8.2 years and 54.8 ± 9.3 years respectively, in the study done by Satyanarayana et al.,(14) which was similar to that of our study population (55.3 ± 5.4 years and 54.8 ± 6.1 years in PDR and no DR group respectively).(14) The study populations of Brazionis et al. (median age of 66.5 years in DR and 65 years in No DR) and Fotiou et al. (median of 68years in DR and 61 years in No DR group) were much older compared to our study population.(11,19)

Gender

Levels of homocysteine are usually higher in men compared to women.(60) Hence, we selected gender-matched controls for each of our cases, to decrease the confounding effect of gender. In our study, there were 33 males and 6 females in both groups. The studies at Brazionis et al., and Fotiou et al., have not looked at gender as a risk factor.(11,19)

Diet

There were 7 cases and 5 controls in our study, who were vegetarian, and there was no statistically significant difference between the groups with respect to dietary habits ($p = 0.53$). Other studies done on this subject have not looked at the dietary habits and preferences of their subjects, which may be important factors in influencing the serum homocysteine levels.(11,13)

Risk factors

Duration of diabetes

Longer duration of diabetes has been associated with several macro and micro vascular complications, including retinopathy.(61) The duration of diabetes (>15 years) was significantly higher in the proliferative retinopathy group (cases) as compared to the no retinopathy group (controls) (35.9% of cases vs. 2.6% of controls; $p < 0.01$) in our study. Similarly, the duration of diabetes in the retinopathy group was significantly higher when compared to the no retinopathy group, in the studies done by Brazionis et al. and Fotiou et al.(11,19) However, the mean duration of diabetes was 11.03 ± 6.9 years vs. 10.16 ± 6.9 years in DR and no DR groups in the study done by Satyanarayana et al., and the difference was not statistically significant ($p = 0.09$).(14)

Fotiou et al., found significantly increasing median (IQR) serum homocysteine levels with increasing duration of diabetes (11.2 µmol/L (8.7 – 13.9 µmol/L) in patients with duration of diabetes ≤ 5 years vs. 16.9 µmol/L (14.8 – 18.9 µmol/L) in patients with duration of diabetes ≥ 16 years; $p < 0.001$, and 12.2 µmol/L (10.0 – 16.2 µmol/L) in patients with duration of diabetes 6 – 15 years vs. 16.9 µmol/L (14.8 – 18.9 µmol/L) in patients with duration of diabetes ≥ 16 years; $p = 0.001$).(19).This study also found a statistically significant difference in serum homocysteine levels between the NPDR and PDR groups (median (IQR) in NPDR group: 15.5 µmol/L (11.8 – 17.4 µmol/L) vs. 18.7 µmol/L (16.5 – 22.0 µmol/L) in the PDR group.(19)

Glycemic control

The mean/ median (with SD/ IQR) HbA1c levels were significantly higher in patients with DR compared to patients without DR in studies done by Satyanarayana et al. (10.3 ± 2.9% vs. 9 ± 2.5%; $p < 0.01$),(14) Brazionis et al. (8.6% (7.1 – 10.2%) vs. 7.6% (6.6 – 8.7%); $p = 0.003$)(11) and Fotiou et al. (7.4% (6.6 – 8.9%) vs. 6.7% (6.0 – 7.6%); $p < 0.001$).(19) In our study, the mean (± SD) HbA1c level was higher among cases as compared to controls (8.6 ± 2.4% vs. 7.7 ± 1.3%; $p = 0.05$) with borderline significance, although there was no significant difference between the two groups with respect to glycemic control. Poor glycemic control (defined as HbA1c > 7%) was present in 64.1% of cases vs. 71.8% of controls; p = 0.47). We also did not find any statistically significant correlation of serum HbA1C levels and homocysteine levels in the two groups (PDR: r = 0.04, p = 0.80 and No DR: r = -0.23, p = 0.16).

Hypertension

The mean ± SD systolic blood pressure was significantly higher in the PDR group as compared to the no DR group (137.4 ± 1.8 mmHg vs. 126.4 ± 16.1 mmHg, p = 0.01) in our study. We could not demonstrate any significant difference in the mean diastolic blood pressure between the two groups (83.0 ± 10.0 mmHg vs. 79.7 ± 8.9 mmHg, p = 0.12), as was seen in the study by Fotiou et al.(19)There was no significant difference in the systolic or diastolic blood pressure between the two groups (DR and no DR) in the study done by Brazionis et al.(11) However, we found that the prevalence of hypertension was significantly higher in cases as compared to the controls in our study (84.6% vs. 51.3%; p < 0.01).

Anemia

Satyanarayana et al. could not demonstrate a significant difference in the mean hemoglobin levels (14.1 ± 2.3 g/dL and 14.3 ± 2.2 g/dL; p = 0.25) between the two groups (DR and no DR) in their study.(14) However, we found that the mean (± SD) hemoglobin level was significantly lower in cases as compared to controls in our study (11.62 ± 1.96 g/dL vs. 13.52 ± 1.62 g/dL; p < 0.001). Similarly, the prevalence of anemia was significantly higher in cases as compared to controls (74.4 % vs. 28.2%; p < 0.001). However, we did not find a statistically significant correlation of hemoglobin levels with homocysteine levels in either of the two groups (PDR: r= -0.19, p = 0.24 and No DR: r = -0.01, p = 0.93).

Hyperlipidemia

The mean (± SD) of serum LDL levels was higher in cases as compared to controls, with borderline significance (112.9 ± 40.0 mg/dL vs. 96.6 ± 32.7 mg/dL; p=0.05), though prevalence of hyperlipidemia (defined as LDL levels > 100 mg/dL or known case of hyperlipidemia receiving treatment) was not significantly different between the two groups (82.1% vs. 66.7%; p= 0.12). Though Satyanarayana et al.,(14) could not find any significant difference with respect to mean LDL levels between the two groups in their study (DR: 117.1 ± 34 mg/dL vs. No DR: 110.7 ± 29.0 mg/dL; p = 0.6), the mean of both groups was above the upper limit of normal. We did not find any statistically significant correlation between LDL and homocysteine levels in either of the two groups in our study (PDR: r = 0.05, p = 0.78 and No DR: r = -0.21, p = 0.20).

Renal dysfunction

In our study, there was significantly increased prevalence of renal dysfunction, as measured by elevated mean creatinine levels (1.75 ± 1.33 mg/dL vs. 0.89 ± 0.27 mg/dL; p < 0.001) and declining mean ± SD estimated GFR (62.84 ± 33.01 ml/min/1.73m^2 vs. 92.43 ± 23.59 ml/min/1.73m^2; p < 0.001) in the PDR group as compared to the no DR group. Similarly, there were significantly elevated serum creatinine levels in the retinopathy group as compared to the no retinopathy group in the study done by Fotiou et al.(19)

The prevalence of renal dysfunction was also significantly higher among the cases as compared to the controls in our study (41% vs. 7.7 %; $p < 0.001$). However, there was no statistically significant correlation of eGFR with serum homocysteine levels in either of the two groups in our study (PDR: $r = -0.22$, $p = 0.18$ and no DR: $r = -0.27$, $p = 0.10$), though there was a statistically significant positive correlation of serum creatinine with homocysteine levels in the no DR group ($r = 0.32$, $p = 0.046$).

Body Mass Index

The BMI of patients with DR and no DR was 24.3 ± 4.5 kg/m^2 and 25.2 ± 4.2 kg/m^2 respectively in the study done by Satyanarayana et al., (14) which was almost similar to that of our study population (26.3 ± 4.1 kg/m^2 and 27.0 ± 4.2 kg/m^2 respectively, $p = 0.5$). The median BMI of patients in the DR and no DR groups was higher (median of 28 kg/m^2 in DR and 30 kg/m^2 in no DR) in the study by Brazionis et al.(11) and also in the study by Fotiou et al. (median of 29.2 kg/m^2 in DR and 27 kg/m^2 in no DR).(19)

Drugs affecting homocysteine

Patients with diabetes are usually on several drugs - oral hypoglycemic drugs like metformin, statins as cholesterol reducing agents, diuretics for renal dysfunction, and several multivitamin preparations, which contain vitamin B12 and folic acid, all of which may influence serum homocysteine levels.(62) In the study done by Satyanarayana et al., although there was a significant negative correlation of the serum homocysteine levels with folate and vitamin B12 levels, there was no significant correlation with vitamin B1, B2 and B6 levels.(14) In India, the prevalence of multinutrient deficiency, including vitamin B12 deficiency, is quite high, which may have a bearing on serum homocysteine levels.(14) In this study, the lower vitamin B12 and folate levels were significantly associated with increasing homocysteine concentrations.(14)

Fotiou et al. suggested a threshold level of serum vitamin B12 (248 pg/mL), below which there is significant elevation of serum homocysteine with decline in serum B12 levels. In this study, they found a statistically significant negative correlation between serum homocysteine levels and folic acid levels ($r = -0.261$, $p = 0.002$).(19)

Fotiou et al. also found that there was a significantly higher prevalence of hyperhomocysteinemia, increased serum homocysteine levels and significantly lower serum vitamin B12 and folate levels in patients with DR, as compared to patients with no DR.(19)

There were higher number of cases with PDR in our study, who were on multivitamin, vitamin B12, B6, nicotinamide and folate supplementation, compared to the controls. This difference was statistically significant in the case of folate supplementation, with 25.6% of the cases being on folate supplementation, while none of the controls were taking folate supplements ($p = 0.001$). This could have resulted in lower serum homocysteine levels in the cases in our study. We did not assess the serum folate and vitamin B12 levels in our study subjects due to financial constraints.

Metformin is a commonly used oral hypoglycemic drug in diabetes mellitus, but it is known to cause Vitamin B12 deficiency, which may result in elevation of serum homocysteine levels.(62) This could also have affected our results, as there was a significantly higher number of controls taking metformin (36 controls, 92.3%) compared to the cases (18 cases, 46.2%), $p < 0.001$. Sato et al. also found marginally higher homocysteine levels in metformin users as compared with metformin non-users.(63)

HOMOCYSTEINE AND RETINOPATHY

Although a number of studies have been done to study the association of diabetic retinopathy with hyperhomocysteinemia, the results have not always been consistent. Some studies have found a strong association of hyperhomocysteinemia with diabetic retinopathy,(11–14,19,42,55) while others have failed to do so.(15-16) These studies have concluded that hyperhomocysteinemia may not be an independent risk factor for diabetic retinopathy, and have suggested that other condition associated with diabetes, like declining renal dysfunction, may cause elevation of serum homocysteine levels in diabetic patients. (15-16)

Satyanarayana et al. found significantly higher homocysteine levels in the no retinopathy group as compared to the control group without diabetes. They also showed significantly higher serum homocysteine levels in the patients in the diabetic retinopathy group as compared to the no retinopathy group.(14) Similarly, Brazionis et al. showed significantly higher median (IQR) homocysteine level in patients with DR as compared to patients without DR (11.5 µmol/L (10.4– 12.5 µmol/L) vs. 9.6 µmol/L (9.1–10.2 µmol/L), respectively; p=0.001).(11)

In the study done by Fotiou et al., significantly higher median (IQR) homocysteine levels were seen in the DR as compared to the no DR group (16.3 µmol/L (14.7 – 19.8 µmol/L) vs. 11.1 µmol/L (9.5 – 13.6 µmol/L); p < 0.001). The prevalence of hyperhomocysteinemia (Hcy levels more than 15 micromol/L) was also significantly higher in the DR group as compared to patients without DR.(19)

Malagaurnera et al., compared serum homocysteine levels in patients with PDR, NPDR, no DR and healthy controls. There was a significantly higher mean homocysteine level in the PDR group, compared to the no DR group (18.2 ± 5.6 µmol/L vs. 12.1 ± 6.8 µmol/L; p < 0.01). They also found a significant increase in the homocysteine levels with progression of severity of retinopathy. In this study, they found that the odds ratio for hyperhomocysteinemia was 4.2 and 1.2 in the PDR and NPDR groups, respectively.(13)

Hultberg et al., in their study, found significantly elevated mean ± SD homocysteine level in patients with T1DM with PDR (15.0 ± 6.3µmol/L; p < 0.001) when compared with patients with no or minimal DR (10.7 ± 4.3 µmol/L) or controls (11.0 ± 3.4 µmol/L). They also observed that this increased serum homocysteine level was mainly confined to patients with declining renal function (elevated serum creatinine or deranged albumin : creatinine ratio), and that those with no or minimal renal dysfunction had normal serum homocysteine levels.

Hence, they suggested the role of advanced nephropathy in elevating serum homocysteine levels, rather than diabetes or diabetic retinopathy per se.(15)

Agardh et al. found that the mean ± SD serum Hcy level in patients with microalbuminuria (9.1 ± 3.4µmol/L) was not significantly different from patients with normoalbuminuria (8.0 ± 1.7µmol/l). However, patients with clinical signs of renal dysfunction had higher plasma Hcy levels (12.9 ± 5.7 µmol/L; $p < 0.01$) compared to those with microalbuminuria and normoalbuminuria. The authors of this study also could not demonstrate any association between different levels of retinopathy and plasma homocysteine levels.(16)

In a meta-analysis done by Xu et al., including 31 studies and 6394 patients, it was found that the Hcy levels in the blood of patients with DR were higher than that of patients in the control group, although there was statistical heterogeneity among the studies.(46) Xu et al. also observed that the role of hyperhomocysteinemia was probably more significant in T1DM or in mixed (Type1+2) diabetes,(46) rather than in patients with T2DM, who constituted our study population.

The mean plasma levels of homocysteine were 15.87 µmol/L in the PDR group and 13.46 µmol/L in the no DR group in the study done by Goldstein et al.(12) The authors also found significantly increased homocysteine concentrations with increasing severity of DR. The mean plasma levels in patients with PDR (15.86 ± 1.34 µmol/L) were significantly elevated as compared to levels in patients with NPDR (14.56 ± 0.64 µmol/L), no DR (13.46 ± 0.74 µmol/L), and in controls

(11.75 ± 0.24 µmol/L).(12) Brazionis et al. found that, although the levels of homocysteine were significantly higher among the patients with DR, as compared to controls, the difference between the two groups was small (< 2 µmol/L).(11)

In our study, although the mean (± SD) level of serum homocysteine was higher among the cases as compared to the controls, the difference was not statistically significant. (17.98 ± 6.26 µmol/L in cases vs. 17.71 ± 8.17 µmol/L in controls; p = 0.87). Sato et al. found that even an increase in 1 µmol/L in homocysteine level was associated with retinopathy, with odds ratio of 1.26, after adjusting for duration of diabetes and glycemic control.(63) Brazionis et al. also concluded that even a small increase in the serum homocysteine levels, in the order of > 1 µmol/L, can be considered as a useful marker for clinicians to make decisions regarding intensification of treatment of both diabetes and its various co morbidities.(11)

In our study, the mean serum homocysteine levels in both cases (17.98 µmol/L) and controls (17.71 µmol/L) were higher than the upper limit of normal (normal homocysteine concentrations: 5 - 15 µmol /L).(38,47,60) Brazionis et al. have shown lower mean homocysteine levels in both cases (DR) and controls (no DR), when compared to age-appropriate levels.(11)

As per the study protocol, the median homocysteine level of the control group was taken as the cut off to define hyperhomocysteinemia (≥ 16.19 µmol/L). There was no statistically significant difference between the two groups (59.0% vs. 48.7%; p = 0.36), when hyperhomocysteinemia was defined as ≥ 16.19 µmol/L. In view of the heterogeneity in the definition of hyperhomocysteinemia, we tried analyzing our results, taking different cut off values for defining hyperhomocysteinemia. However, we could not find a statistically significant difference between the two groups in our study, with cut off levels at $\geq$ 10 µmol/L (92.3% vs. 89.7%; $p = 1.0$), $\geq$ 12 µmol/L (84.6% vs. 76.9%; $p = 0.39$), and $\geq$ 15 µmol/L; 64.1% vs. 53%; $p = 0.36$).

The presence of significantly increased intake of metformin in the control group (92.3% in controls vs. 46.2% in cases; $p < 0.001$), and significantly increased intake of folate in the cases (25.6 % in cases vs. 10% in controls; $p = 0.001$), could have skewed the results of our study to some extent.

CONCLUSION

1. The prevalence of hyperhomocysteinemia was higher (59%) in the cases with proliferative diabetic retinopathy (PDR), as compared to the controls with no retinopathy (48.7%). However, the difference in the prevalence of hyperhomocysteinemia between the cases and controls was not statistically significant ($p = 0.36$).
2. Although the mean (± SD) level of serum homocysteine was higher among the cases as compared to the controls, the difference was not statistically significant. (17.98 ± 6.26 µmol/L in cases vs. 17.71 ± 8.17 µmol/L in controls; $p = 0.87$).
3. The mean serum levels of homocysteine in both cases with proliferative diabetic retinopathy (17.98 ± 6.26 µmol/L) and in controls with no retinopathy (17.71 ± 8.17 µmol/L) were higher than the upper limit of normal. (normal homocysteine concentration: 5 - 15 µmol /L)
4. Longer duration of diabetes, hypertension, anemia and renal dysfunction, which are known risk factors for progression of diabetic retinopathy, were found to be significantly associated with PDR.

Limitations of the study

1. We calculated the sample size for this study with an odds ratio of 4 in order to detect a clinically significant difference between the two groups. Therefore, the study was not designed to detect smaller differences between the cases and controls.
2. There was a difference between the two groups with respect to the use of many drugs that are known to alter serum homocysteine levels. The use of multivitamins, including vitamin B12 supplementation, was more in the cases as compared to the controls. This could have lowered the serum homocysteine levels in the cases. There was a statistically significant difference between the two groups with respect to the use of metformin and folate supplementation, with the use of metformin being more common in controls, and folate supplementation being more common in cases. This could have further undermined the difference in serum homocysteine levels between the two groups.
3. We did not estimate the serum levels of vitamin B12 and folate in our study subjects due to financial constraints, and therefore, we could not find out if stheir serum homocysteine levels were influenced by the vitamin B12 and folate levels.

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APPENDIX 1

IRB APPROVAL FORM

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA.

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Centre,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho, MS Ortho, DNB Ortho
Chairperson, Research Committee & Principal

Dr. Nihal Thomas,
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Edin), FRCP (Glasg)
Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

November 17, 2014

Dr. Prabha Gupta
PG Registrar
Department of Ophthalmology
Christian Medical College
Vellore 632 002

Sub: **Fluid Research Grant Project:**
Role of homocysteine in proliferative diabetic retinopathy: a case-control study.
Dr. Prabha Gupta, PG Registrar, Dr. Sheeja Susan John, Dr. Deepa John, Ophthalmology, Mrs. Grace Rebekah J, Biostatistics, CMC, Vellore.

Ref: IRB Min No. 9130 dated 12.11.2014

Dear Dr. Prabha Gupta,

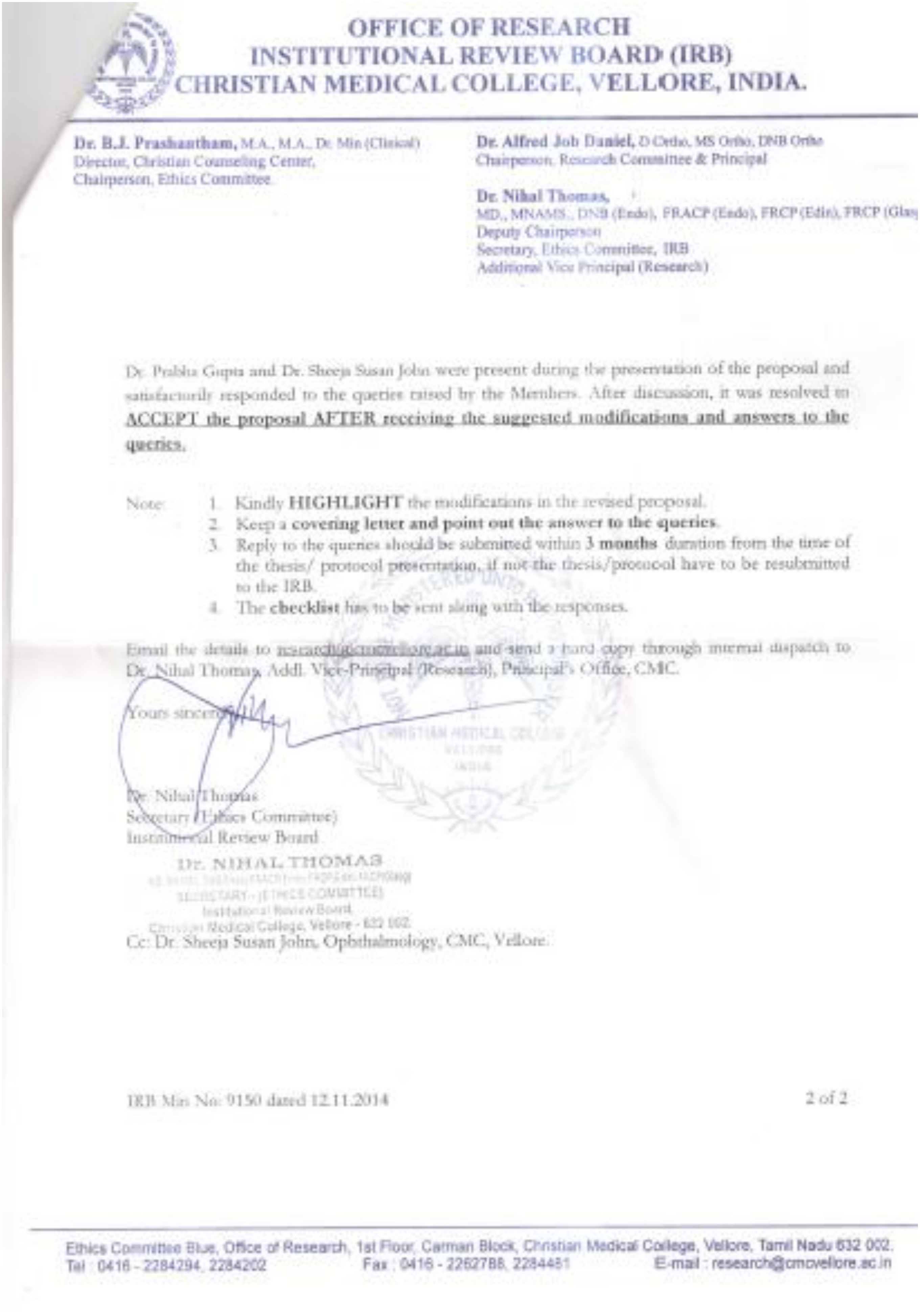
The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "Role of homocysteine in proliferative diabetic retinopathy: a case-control study." on November 12th 2014. I am quoting below the minutes of the meeting.

The Committee raised the following queries:

a) Please give the detailed methodology.
b) Please acknowledge the text done from other sources.
c) Metformin will be present in both groups, this will have an impact on both arms of the study. Vitamin B12 deficiency will be covered particularly in the arm with proliferative retinopathy - so Vitamin B12 levels should be measured, to make this a publishable study.
d) Which competitive immunoassay are you using?
e) Liaise with clinical biochemistry during the study. The type of tube needs to be specified.
f) Other factors like renal disease and autonomic dysfunction are likely to precipitate vitamin B12 deficiency.

1 of 2

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel : 0416 - 2284294, 2284202 Fax : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in



OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA.

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
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Dr. Nihal Thomas,
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Edin), FRCP (Glas
Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

Dr. Prabha Gupta and Dr. Sheeja Susan John were present during the presentation of the proposal and satisfactorily responded to the queries raised by the Members. After discussion, it was resolved to **ACCEPT the proposal AFTER receiving the suggested modifications and answers to the queries.**

Note:
1. Kindly **HIGHLIGHT** the modifications in the revised proposal.
2. Keep a **covering letter and point out the answer to the queries**.
3. Reply to the queries should be submitted within **3 months** duration from the time of the thesis/ protocol presentation, if not the thesis/protocol have to be resubmitted to the IRB.
4. The **checklist** has to be sent along with the responses.

Email the details to research@cmcvellore.ac.in and send a hard copy through internal dispatch to Dr. Nihal Thomas, Addl. Vice-Principal (Research), Principal's Office, CMC.

Yours sincerely,

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

DR. NIHAL THOMAS
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board
Christian Medical College, Vellore - 632 002.

Cc: Dr. Sheeja Susan John, Ophthalmology, CMC, Vellore.

IRB Min No: 9150 dated 12.11.2014

2 of 2

APPENDIX 2

CLINICAL RESEARCH FORM

Homocysteine And Retinopathy in Diabetes (HARD)

Case/ Control

Study Serial No: Date:

Schell Hospital No: CMCH No:

Name:

Age (years): Ht: ………cm Wt…….Kg

Gender: 1. Male 2. Female

State of Residence: 1.Tamil Nadu 2. Outside Tamil Nadu

Phone No:

Clinical Diagnosis: 1. No DR 2. PDR
(ocular)
Other details (if any):

HISTORY:

Duration of diabetes: 1. <5yrs 2. 5-10yrs 3. >10-15yrs 4. >15yrs (specify duration)

Diet 1.Vegetarian 2. Non-vegetarian

History of:

- Hypertension 0. No 1.Yes
- Hyperlipidemia 0. No 1.Yes
- Smoking 0. No 1.Yes (specify)
- Alcoholism 0. No 1.Yes (specify)
- Anemia 0. No 1.Yes
- Kidney Disease 0. No 1.Yes
- Malabsorption syndromes 0. No 1. Yes (specify)

Drug history: (specify drug, dosage & duration of treatment)

Oral contraceptive pills	0. No	1. Yes
Diuretics	0. No	1. Yes
Metformin	0. No	1. Yes
Fibrates	0. No	1. Yes
MVT	0. No	1. Yes
Vitamin B12	0. No	1. Yes
Folate	0. No	1. Yes
Vitamin B6	0. No	1. Yes
Nicotinic acid	0. No	1. Yes
Anti-epileptics	0. No	1. Yes
Levodopa	0. No	1. Yes
Sulfasalazine	0. No	1. Yes
Trimethoprim	0. No	1. Yes
Pyrimethamine	0. No	1. Yes
Methotrexate	0. No	1. Yes
Proton pump inhibitors	0. No	1. Yes
Histamine 2 receptor antagonists	0. No	1. Yes
Any other drug (details)		

Investigations and results:

BMI: ….. 1.Underweight 2. Normal 3.Overweight 4.Obese

AC/PC: …………mg/dl 1.Normal 2.Increased

HbA1C: ……% 1.Normal 2.Increased

Glycemic control: 1. Good 2. Poor

Blood Pressure: …………mmHg 1.Normal 2.Increased

Hyperlipidemia: (values)…. 0. No 1.Yes

Creatinine: …………mg/dl 1.Normal 2.Increased

eGFR: ……

Renal dysfunction: 0. No 1. Yes

Hb: …… g/dl
Anemia: 0. No 1. Yes
Homocysteine: ……..(µmol/l)

APPENDIX 3
ENGLISH INFORMATION SHEET AND CONSENT FORM

Protocol No:

Homocysteine And Retinopathy in Diabetes
(H A R D)
Information Sheet - Cases

Name of participant:

You are invited to take part in this study. The information in this document is meant to help you decide whether or not to take part in the study. Please feel free to ask if you have any queries or concerns.

What is the study about?

Diabetic Retinopathy is a disease of the eye caused by damage to the blood vessels of the retina (the nerve at the back of the eye). This disease is a complication of diabetes, and progresses from mild ‘non-proliferative’ stage of retinopathy to ‘proliferative’ stage of retinopathy, when the patient develops new blood vessels, which proliferate in the retina, and cause bleeding into the eye and detachment of the retina. This results in severe visual impairment and blindness. Proliferative diabetic retinopathy is an important cause of blindness in adults. There are several known risk factors for the disease like high blood pressure, high cholesterol levels etc. Increase in blood levels of homocysteine (a molecule found in blood) has also been suspected to cause this disease. Increase in blood levels of this molecule is also known to cause other systemic diseases like heart attack and stroke. In this study, we will check the blood levels of homocysteine of the participants, in order to find out if increased blood level of homocysteine is associated with proliferative diabetic retinopathy.

If you take part, what will you have to do?

If you take part in the study, you will be asked a few questions, which are relevant to the study. You will have a routine eye examination. You will also be asked to come in the morning on another day, after overnight fasting, for blood collection, in order to check your blood levels of homocysteine, along with measurement of blood pressure, height and weight. Other routine blood and eye tests that are needed for the diagnosis and treatment of your eye disease will also be done as required.

Are there any risks for you if you take part in the study?

In addition to the routine eye tests required for the management of your eye disease, participation in the study only involves testing your blood sample for homocysteine. We do not expect any injury to happen to you as a result of participation in this study; but if you do develop any side effects or problems due to the study, these will be treated at no cost to you. However, we are unable to provide any monetary compensation.

Do you have to pay?

You will have to pay only for the tests that are required for the routine evaluation and treatment of your eye disease. The testing of blood homocysteine level for the study will be done free of cost.

What are the benefits to you if you take part in the study?

If you participate in the study,
1. You will be screened for high blood levels of homocysteine, which may have played a role in the development of your eye disease.
2. In case you are found to have abnormal blood levels of these molecules, you will be referred to specialist doctors for further evaluation and appropriate treatment.
3. Early detection and treatment of such abnormal blood levels may help in preventing the occurrence of systemic diseases like stroke and heart attack.

What are the possible benefits to other people?

The results of this study may provide benefits to the society in terms of advancement of medical knowledge, disease prevention and therapeutic benefit to future patients. We hope that this study will help us to understand this disease better. In the future, this may help us to devise strategies to prevent or delay the development of this eye disease in diabetic patients, as well as to prevent the occurrence of systemic complications like stroke and heart attack.

Can you decide not to participate?

Your participation in this study is entirely voluntary and you are also free to decide to withdraw permission to participate in this study. If you do so, this will not affect your usual treatment at this hospital in any way. Your doctor will still take care of you and you will not lose any benefits to which you are entitled.

Will your personal details be kept confidential?

The results of this study may be published in a medical journal, but you will not be identified by name in any publication or presentation of results. However, your medical notes may be reviewed by people associated with the study, without your additional permission, should you decide to participate in this study.

If you have any further questions, you may contact Dr. Prabha Gupta or Dr. Sheeja Susan John or Dr. Deepa John (Tel: 0416 2281201) or email:
drprabhagupta@gmail.com

Homocysteine And Retinopathy in Diabetes
(H A R D)
Information Sheet - Controls

Name of participant:

You are invited to take part in this study. The information in this document is meant to help you decide whether or not to take part in the study. Please feel free to ask if you have any queries or concerns.

What is the study about?

Diabetic Retinopathy is a disease of the eye caused by damage to the blood vessels of the retina (the nerve at the back of the eye). This disease is a complication of diabetes, and progresses from mild 'non-proliferative' stage of retinopathy to 'proliferative' stage of retinopathy, when the patient develops new blood vessels, which proliferate in the retina, and cause bleeding into the eye and detachment of the retina. This results in severe visual impairment and blindness. Proliferative diabetic retinopathy is an important cause of blindness in adults. There are several known risk factors for the disease like high blood pressure, high cholesterol levels etc. Increase in blood levels of homocysteine (a molecule found in blood) has also been suspected to cause this disease. Increase in blood levels of this molecule is also known to cause other systemic diseases like heart attack and stroke. In this study, we will check the blood levels of homocysteine of the participants, in order to find out if increased blood level of homocysteine is associated with proliferative diabetic retinopathy.

To understand this well, we need to look for high blood levels of homocysteine in diabetics who do not have diabetic retinopathy. The information obtained by doing this will be compared with the results obtained by doing the same tests on diabetic patients who have proliferative diabetic retinopathy. This comparison will help us to understand the role of increased blood levels of homocysteine in this disease.

If you take part, what will you have to do?

If you take part in the study, you will be asked a few questions, which are relevant to the study. You will have a routine eye examination. You will also be asked to come in the morning on another day, after overnight fasting, for blood collection, in order to check your blood levels of homocysteine, along with measurement of blood pressure, height, and weight. Other routine blood tests that are needed for the management of diabetes will also be done as required.

Are there any risks for you if you take part in the study?
Participation in the study only involves a routine eye examination that is done for all patients in the eye hospital, and collection of a blood sample for tests. We do not expect any injury to happen to you as a result of participation in this study; but if you do develop any side effects or problems due to the study, these will be treated at no cost to you. However, we are unable to provide any monetary compensation.

Do you have to pay?
You will have to pay only for the routine tests that are required for the management of diabetes. The testing of blood homocysteine level for the study will be done free of cost.

What are the benefits to you if you take part in the study?
If you participate in the study,

1. You will be screened for high blood levels of homocysteine.
2. In case you are found to have abnormal blood levels of these molecules, you will be referred to specialist doctors for further evaluation and appropriate treatment.
3. Early detection and treatment of such abnormal blood levels may help in preventing the occurrence of systemic diseases like stroke and heart attack.

What are the possible benefits to other people?
The results of this study may provide benefits to the society in terms of advancement of medical knowledge, disease prevention and therapeutic benefit to future patients. We hope that this study will help us to understand this disease better. In the future, this may help us to devise strategies to prevent or delay the development of this eye disease in diabetic patients, as well as to prevent the occurrence of systemic complications like stroke and heart attack.

Can you decide not to participate?
Your participation in this study is entirely voluntary and you are also free to decide to withdraw permission to participate in this study. If you do so, this will not affect your usual treatment at this hospital in any way. Your doctor will still take care of you and you will not lose any benefits to which you are entitled.

Will your personal details be kept confidential?
The results of this study may be published in a medical journal, but you will not be identified by name in any publication or presentation of results. However, your medical notes may be reviewed by people associated with the study, without your additional permission, should you decide to participate in this study.

If you have any further questions, you may contact Dr. Prabha Gupta or Dr. Sheeja Susan John or Dr. Deepa John (Tel: 0416 2281201) or email:
drprabhagupta@gmail.com

Homocysteine And Retinopathy in Diabetes
(H A R D)
Consent form

Study Number: ____________
Subject's Initials: __________________ Subject's Name:
____
Date of Birth / Age: ___________________________

(Subject)

(i) I confirm that I have read and understood the information sheet dated ____________ for the above study and have had the opportunity to ask questions.
(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.
(iii) I understand that the researchers conducting this study, others working on the researchers' behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published.
(iv) I agree not to restrict the use of any data or results that arise from this study, provided such a use is only for scientific purpose(s).
(v) I agree to take part in the above study.

Signature (or Thumb impression) of the Subject/ Legally Acceptable Representative:
Date: _____/_____/______
Signatory's Name: _________________________________ Signature:
or

Signature of the Investigator: ________________________

Date: _____/_____/______

Study Investigator's Name: _________________________

Signature of the Witness: ___________________________

Date: _____/_____/_______

Name &Address of witness ……………………….

APPENDIX 4
HINDI INFORMATION SHEET AND CONSENT FORM

प्रोटोकॉल नं.
होमोसिसटीन एण्ड रेटिनोपैथी
(एच ए आर डी)
सूचना पत्र निरीक्षण

प्रतिभागी का नाम

आपको इस अध्ययन में भाग लेने के लिए आमंत्रित किया जाता है। इस दस्तावेज में दी गई जानकारी आपको इस अध्ययन में भाग लेने अथवा न लेने के निर्णय में सहायक होगी। आप किसी भी प्रश्न अथवा जिज्ञासा को पूछने के लिए स्वतंत्र महसूस करें।

यह अध्ययन किस विशय में है?

मधुमेह रेटिनोपैथी रेटिना (आंख का पर्दा/तंत्रिका) की रक्त वहिकाओं को नुकसान की वजह से होने वाली आंख की एक बीमारी है। यह बीमारी मधुमेह से होने वाली एक समस्या है। यह धीरे धीरे रेटिना में नई (असामान्य) रक्त वहिकाओं के उत्पन्न होने से गंभीर अवस्था प्रोलिफरेटिव डायबिटिक रेटिनापैथी (पी डी आर) में पहुॅच जाती है। इस बीमारी मे रेटिना की रक्त वहिकाओं के फटने से आंखो

में खून बहता है और इस वजह से मरीज को दृश्टि में कमी और अंधापन आ जाता है।
पी डी आर व्यस्कों में अंधेपन का प्रमुख कारण है। जैसे कि उच्च रक्त चाप, उच्च कोलेस्ट्रॉल मधुमेह के ज्ञात जोखिम कारक है, उसी प्रकार होमोसिसटीन अणु के रक्त स्तर में वृद्धि होना भी इस रोग के लिए एक संदेहकारक है। इस अणु का उच्च रक्त स्तर हृदय रोग और स्ट्रोक का भी एक कारक है। इस अध्ययन में हम प्रतिभागियों के रक्त में होमोसिसटीन का स्तर मालूम कर, यह तय करने की कोशिश करेंगे कि होमोसिसटीन का उच्च स्तर पी डी आर से किस प्रकार जुडा हुआ है या नहीं जुडा है।

यदि आप भाग लेते है तो आपको क्या करना होगा ?

अगर आप इस अध्ययन में भाग लेते है तो आपको अध्ययन से जुडे कुछ सवालों का जवाब देना होगा। आपका नियमित नेत्र परीक्षण होगा, साथ ही आपका रक्तचाप, ऊंचाई एवम् वजन का माप होगा। होमोसिसटीन स्तर की जांच के रक्त संग्रह के लिए रातभर उपवास कर, एक और दिन सुबह आना होगा। आवश्यकता अनुसार आपकी आंखों के परीक्षण और इलाज के लिए दूसरी जांचे की जाऐंगी।

आपके अध्ययन में भाग लेने पर आपको कोई हानि हो सकती है क्या ?

इस अध्ययन से भाग लेने पर नियमित नेत्र परीक्षण के साथ होमोसिसटीन स्तर के लिए रक्त परीक्षण किया जाएगा। हमारे अनुसार इस अध्ययन में भाग लेने पर आपको किसी प्रकार की हानि की आशंका नहीं है फिर भी यदि आपको कोई अध्ययन से जुडी समस्या/परेशानी होती है, तो उसका इलाज मूलरूप से निःशुल्क किया जाएगा।

हालांकि हम किसी भी प्रकार की मुआवजा राशि प्रदान करने में असमर्थ होंगे।

क्या आपको अध्ययन हेतु भुगतान करना पड़ेगा ?

आपको केवल नियमित नेत्र परीक्षण एवं मधुमेह से संबंधित रक्त परीक्षण का खर्च उठाना पड़ेगा अध्ययन के लिए रक्त होमोसिसटीन स्तर निःशुल्क किया जाएगा।

आपके अध्ययन में भाग लेने पर आपको क्या लाभ हैं ?

यदि आप अध्ययन में भाग लेते है तो

1. आपके रक्त में होमोसिसटीन स्तर की जांच की जाएगी, जिसका संबंध नेत्र रोग की तीव्रता से हो सकता है।

2. यदि आपके रक्त में होमोसिसटीन का उच्च स्तर पाया जाता है तो आपको आगे के मूल्यांकन एवं उचित उपचार के लिए विशेशज्ञ डॉक्टर के पास भेजा जाएगा।
3. होमोसिसटीन के उच्च रक्त स्तर का जल्दी पता लगने से अन्य बीमारियां जैसे दिल का दौरा तथा लकवा (स्ट्रोक) को रोकने में मदद मिल सकती है।

अन्य लोगों के लिए इस अध्ययन से क्या लाभ है ?

इस अध्ययन के परिणाम भविश्य में चिकित्सा ज्ञान, बीमारी की रोकथाम और चिकित्सीय लाभ की उन्नति से समाज की लाभ प्रदान कर सकते है। हमें उम्मीद है कि इस अध्ययन से इस बीमारी को बेहतर समझने में मदद मिलेगी, तथा भविश्य में मधुमेह के रोगियों में इस प्रकार के नेत्र रोग को बढने से रोकने का प्रयास किया जा सकेगा, साथ ही दूसरी बीमारियां जैसे लकवा दिल का दौरा को भी रोका जा सकेगा।

क्या आप भाग लेने से इंकार कर सकते है ?

इस अध्ययन में आपकी भागीदारी पूर्णतः स्वैच्छिक है। आप इस अध्ययन में भाग लेने की अनुमति को वापस लेने के लिए पूर्णतः स्वतंत्र है। यदि आप अनुमति वापस लेते है तो यह किसी भी तरह से आपके नियमित परीक्षण व उपचार को प्रभावित नहीं करेगा। आपके डॉक्टर आपकी उसी तरह ख्याल रखेंगे जिसके आप हकदार है।

क्या आपकी व्यक्तिगत जानकारी को गोपनीय रखा जाएगा ?

इस अध्ययन के परिणाम एक मेडिकल जर्नल में प्रकाशित किए जा सकते है लेकिन आपके परिणामों के प्रकाशन या प्रस्तुति में आपके नाम की गोपनियता बरकरार रखी जाएगी। यदि आप अध्ययन के लिए अनुमति देते है तो आपकी चिकित्सा से जुड़े दस्तावेज अध्ययन में भाग लेने वाले सहयोगी, बिना आपकी अनुमति के हेतु उपयेाग कर सकते है।

यदि आपको इस विशय में कोई जानकारी चाहिए या आपका कोई प्रश्न है तो आप डॉ. प्रभा गुप्ता/डॉ शीजा सूसन जॉन/डॉ दीपा जॉन से सम्पर्क कर सकते है।

दूरभाश 04162281201 ईमेल कतचतंईींहनचजं/हउंपसण्बवउ

होमोसिसटीन एण्ड रेटिनापैथी इन डायबिटीज

(एच ए आर डी)

सहमति पत्र

अध्ययन संख्या

प्रतिभागी के प्रथमाक्षर...........

प्रतिभागी का नाम ....

जन्म/आयु की तिथि

प्रतिभागी..

1.मैं यह घोशणा करता हॅू कि मैंने उपरोक्त अध्ययन से संबंधित जानकारी को पढ़ा व पूर्णतः समझा लिया है, तथा इस अध्ययन से संबंधित प्रश्न पूछने का भी अवसर मिला है।

2.अध्ययन में मेरी भागीदारी पूर्णतः स्वैच्छिक है तथा मैं बिना कोई कारण बताए किसी भी समय अपनी अनुमति वापस लेने के लिए स्वतंत्र हॅू, इसके उपरांत मेरे नियमित उपचार पर कोई प्रभाव नहीं पड़ेगा।

3.मैं यह अच्छी तरह समझता हॅू कि इस अध्ययन से जुड़े शोधकर्ताओं, अनुसंधानकर्ताओं तथा अन्य सहयोगियें को मेरी अनुमति बिना वर्तमान तभा भविश्य में अध्ययन के लिए मेरी चिकित्सा से जुड़े दस्तावेजों को उपयोग करने की पूर्ण स्वतंत्रता होगी, हालांकि मेरी पहचान को हमेशा गुप्ता रखा जाएगा।

4.मैं अपनी सहमति व्यक्त करता हूॅ कि इस अध्ययन से जुड़े कोई भी दस्तावेज/परिणाम वैज्ञानिक उद्देश्य से प्रेरित रिसर्च/अध्ययन के लिए उपयेाग किए जा सकते है।

5.मैं उपरोक्त अध्ययन में भाग लेने पर सहमत हूॅ ।

प्रतिभागी के हस्ताक्षर/कानूनी तौर पर स्वीकार्य प्रतिनिधि के हस्ताक्षर (अंगूठे का निशान)

हस्ताक्षरकर्ता का नाम .... या

दिनांक ....

अध्ययनकर्ता के हस्ताक्षर ....

दिनांक ....

अध्ययन जांचकर्ता का नाम ....

ग्वाह के हस्ताक्षर ....

दिनांक...

ग्वाह का नाम व पता ....

प्रोटोकॉल नं.
होमोसिसटीन एण्ड रेटिनोपैथी
(एच ए आर डी)
सूचना पत्र नियंत्रण

प्रतिभागी का नाम

आपको इस अध्ययन में भाग लेने के लिए आमंत्रित किया जाता है। इस दस्तावेज में दी गई जानकारी आपको इस अध्ययन में भाग लेने अथवा न लेने के निर्णय में सहायक होगी। आप किसी भी प्रश्न अथवा जिज्ञासा को पूछने के लिए स्वतंत्र महसूस करें।

यह अध्ययन किस विशय में है?

मधुमेह रेटिनोपैथी रेटिना (आंख का पर्दा/तंत्रिका) की रक्त वहिकाओं को नुकसान की वजह से होने वाली आंख की एक बीमारी है। यह बीमारी मधुमेह से होने वाली एक समस्या है। यह धीरे धीरे रेटिना में नई (असामान्य) रक्त वहिकाओं के उत्पन्न होने से गंभीर अवस्था प्रोलिफरेटिव डायबिटिक रेटिनापैथी (पी डी आर) में पहुॅच जाती है। इस बीमारी मे रेटिना की रक्त वहिकाओं के फटने से आंखो में खून बहता है और इस वजह से मरीज को दृश्टि में कमी और अंधापन आ जाता है।

पी डी आर व्यस्कों में अंधेपन का प्रमुख कारण है। जैसे कि उच्च रक्त चाप, उच्च कोलेस्ट्रॉल मधुमेह के ज्ञात

जोखिम कारक है, उसी प्रकार होमोसिसटीन अणु के रक्त स्तर में वृद्धि होना भी इस रोग के लिए एक संदेहकारक है। इस अणु का उच्च रक्त स्तर हृदय रोग और स्ट्रोक का भी एक कारक है। इस अध्ययन में हम प्रतिभागियों के रक्त में होमोसिसटीन का स्तर मालूम कर, यह तय करने की कोशिश करेंगे कि होमोसिसटीन का उच्च स्तर पी डी आर से किस प्रकार जुडा हुआ है या नहीं जुडा है।

इस बात को अच्छी तरह से समझने के लिए हम मधुमेह के रोगियो में जिनको रेटिनोपैथी नही है तथा जिनमें प्रोलिफरेटिव रेटिनोपैथी है उनके रक्त में होमोसिसटीन स्तर की तुलना करेंगे।

इस तुलना में इस रोग में रक्त में होमोसिसटीन स्तर की वृद्धि की भूमिका को समझने के लिए हमें मदद मिलेगी।

यदि आप भाग लेते है तो आपको क्या करना होगा ?

अगर आप इस अध्ययन में भाग लेते है तो आपको अध्ययन से जुडे कुछ सवालों का जवाब देना होगा। आपका नियमित नेत्र परीक्षण होगा, साथ ही आपका रक्तचाप, ऊंचाई एवम् वजन का माप होगा। होमोसिसटीन स्तर की जांच के रक्त संग्रह के लिए रातभर उपवास कर, एक और दिन सुबह आना होगा। आवश्यकता

अनुसार आपकी आंखों के परीक्षण और इलाज के लिए दूसरी जांचे की जाऐंगी।

आपके अध्ययन में भाग लेने पर आपको कोई हानि हो सकती है क्या ?

इस अध्ययन से भाग लेने पर नियमित नेत्र परीक्षण के साथ होमोसिसटीन स्तर के लिए रक्त परीक्षण किया जाएगा। हमारे अनुसार इस अध्ययन में भाग लेने पर आपको किसी प्रकार की हानि की आशंका नहीं है फिर भी यदि आपको कोई अध्ययन से जुडी समस्या/परेशानी होती है, तो उसका इलाज मूलरूप से निःशुल्क किया जाएगा।

हालांकि हम किसी भी प्रकार की मुआवजा राशि प्रदान करने में असमर्थ होंगे।

क्या आपको अध्ययन हेतु भुगतान करना पड़ेगा ?

आपको केवल नियमित नेत्र परीक्षण एवं मधुमेह से संबंधित रक्त परीक्षण का खर्च उठाना पड़ेगा अध्ययन के लिए रक्त होमोसिसटीन स्तर निःशुल्क किया जाएगा।

आपके अध्ययन में भाग लेने पर आपको क्या लाभ हैं ?

यदि आप अध्ययन में भाग लेते है तो

4. आपके रक्त में होमोसिसटीन स्तर की जांच की जाएगी ।
5. यदि आपके रक्त में होमोसिसटीन का उच्च स्तर पाया जाता है तो आपको आगे के मूल्यांकन एवं उचित उपचार के लिए विशेशज्ञ डॉक्टर के पास भेजा जाएगा।
6. होमोसिसटीन के उच्च रक्त स्तर का जल्दी पता लगने से अन्य बीमारियां जैसे दिल का दौरा तथा लकवा (स्ट्रोक) को रोकने में मदद मिल सकती है।

अन्य लोगों के लिए इस अध्ययन से क्या लाभ है ?

इस अध्ययन के परिणाम भविश्य में चिकित्सा ज्ञान, बीमारी की रोकथाम और चिकित्सीय लाभ की उन्नति से समाज की लाभ प्रदान कर सकते है। हमें उम्मीद है कि इस अध्ययन से इस बीमारी को बेहतर समझने में मदद मिलेगी, तथा भविश्य में मधुमेह के रोगियों में इस प्रकार के नेत्र रोग को बढने से रोकने का प्रयास किया जा सकेगा, साथ ही दूसरी बीमारियां जैसे लकवा दिल का दौरा को भी रोका जा सकेगा।

क्या आप भाग लेने से इंकार कर सकते है ?

इस अध्ययन में आपकी भागीदारी पूर्णतः स्वैच्छिक है। आप इस अध्ययन में भाग लेने की अनुमति को वापस लेने के लिए पूर्णतः स्वतंत्र है। यदि आप अनुमति वापस लेते है तो यह किसी भी तरह से आपके नियमित

परीक्षण व उपचार को प्रभावित नहीं करेगा। आपके डॉक्टर आपकी उसी तरह ख्याल रखेंगे जिसके आप हकदार है।

क्या आपकी व्यक्तिगत जानकारी को गोपनीय रखा जाएगा ?

इस अध्ययन के परिणाम एक मेडिकल जर्नल में प्रकाशित किए जा सकते है लेकिन आपके परिणामों के प्रकाशन या प्रस्तुति में आपके नाम की गोपनियता बरकरार रखी जाएगी। यदि आप अध्ययन के लिए अनुमति देते है तो आपकी चिकित्सा से जुड़े दस्तावेज अध्ययन में भाग लेने वाले सहयोगी, बिना आपकी अनुमति के हेतु उपयेाग कर सकते है।

यदि आपको इस विशय में कोई जानकारी चाहिए या आपका कोई प्रश्न है तो आप डॉ. प्रभा गुप्ता/डॉ शीजा सूसन जॉन/डॉ दीपा जॉन से सम्पर्क कर सकते है।

दूरभाश 04162281201 ईमेल कतचतंईंहनचजं/हउंपसण्बवउ

होमोसिसटीन एण्ड रेटिनापैथी इन डायबिटीज

(एच ए आर डी)

सहमति पत्र

अध्ययन संख्या

प्रतिभागी के प्रथमाक्षर............

प्रतिभागी का नाम ....

जन्म/आयु की तिथि

प्रतिभागी..

6.मैं यह घोशणा करता हूॅ कि मैंने उपरोक्त अध्ययन से संबंधित जानकारी को पढ़ा व पूर्णतः समझा लिया है, तथा इस अध्ययन से संबंधित प्रश्न पूछने का भी अवसर मिला है।

7.अध्ययन में मेरी भागीदारी पूर्णतः स्वैच्छिक है तथा मैं बिना कोई कारण बताए किसी भी समय अपनी अनुमति वापस लेने के लिए स्वतंत्र हूॅ, इसके उपरांत मेरे नियमित उपचार पर कोई प्रभाव नहीं पड़ेगा।

8.मैं यह अच्छी तरह समझता हूॅ कि इस अध्ययन से जुड़े शोधकर्ताओं, अनुसंधानकर्ताओं तथा अन्य सहयोगियें को मेरी अनुमति बिना वर्तमान तभा भविश्य में अध्ययन के लिए मेरी चिकित्सा से जुड़े दस्तावेजों को उपयोग करने की पूर्ण स्वतंत्रता होगी, हालांकि मेरी पहचान को हमेशा गुप्ता रखा जाएगा।

9. मैं अपनी सहमति व्यक्त करता हूँ कि इस अध्ययन से जुड़े कोई भी दस्तावेज/परिणाम वैज्ञानिक उद्देश्य से प्रेरित रिसर्च/अध्ययन के लिए उपयोग किए जा सकते है।

10. मैं उपरोक्त अध्ययन में भाग लेने पर सहमत हूँ ।

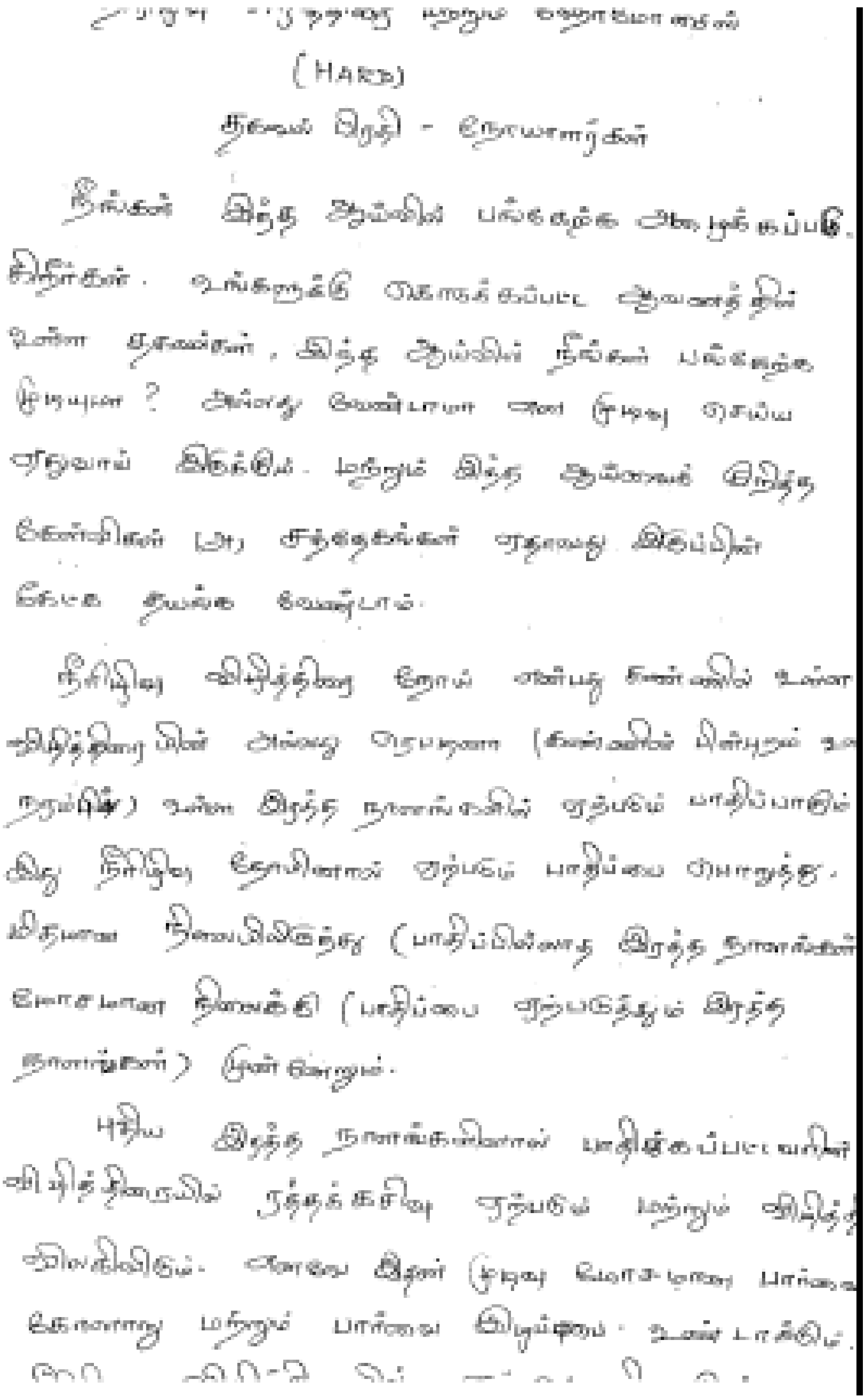
प्रतिभागी के हस्ताक्षर/कानूनी तौर पर स्वीकार्य प्रतिनिधि के हस्ताक्षर (अंगूठे का निशान)

हस्ताक्षरकर्ता का नाम .... या
दिनांक ....

अध्ययनकर्ता के हस्ताक्षर ....
दिनांक ....
अध्ययन जांचकर्ता का नाम ....
ग्वाह के हस्ताक्षर ....
दिनांक...
ग्वाह का नाम व पता ....

APPENDIX 5:

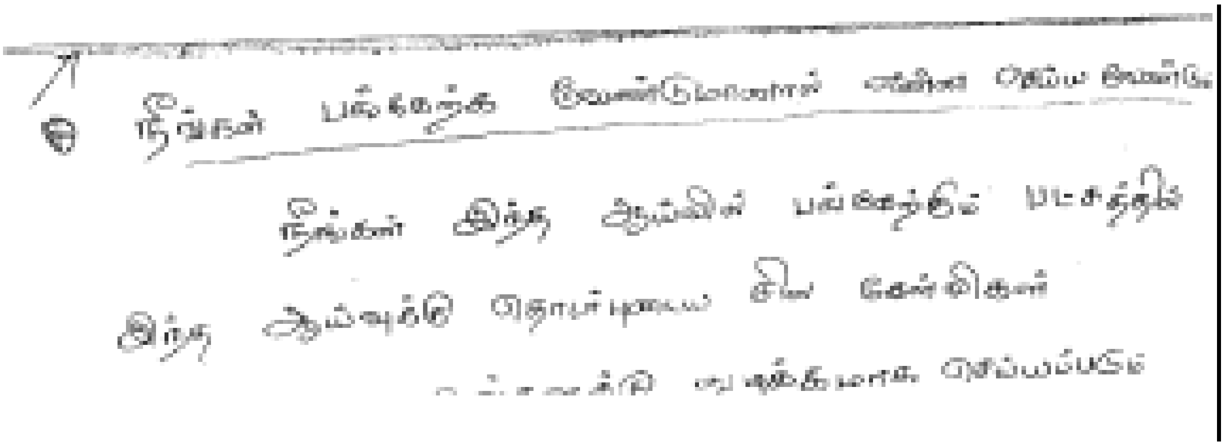
TAMIL IFORMATION SHEET AND CONSENT FORM



① இதை நன்றாக புரிந்து கொள்ள வேண்டும் என்றால் நீரிழிவு விழித்திரை நோய் இல்லாதவர்களின் உடைய இரத்தத்தில் உள்ள மூலக்கூறின் அளவு அறிந்து கொள்ள வேண்டும். இந்த அளவுகளையும் நீரிழிவு விழித்திரை நோய் உள்ளவர்களின் உடைய இரத்தத்தில் உள்ள மூலக்கூறின் அளவையும் ஒப்பிட்டு பார்க்க வேண்டும். இப்படி ஒப்பிட்டு பார்ப்பதன் மூலம் இந்த நோயினால் பாதிக்கப்பட்டவரின் இரத்தத்தில் உள்ள மூலக்கூறின் பங்கை புரிந்து கொள்ள முடியும்.

[illegible] [illegible] இரத்தத்தில் உள்ள [illegible] மூலக்கூறு அதிகமாக இருப்பவர்கள் மாரடைப்பு மற்றும் பக்கவாதம் போன்ற நோய்கள் ஏற்பட காரணமாகும்.

இந்த ஆய்வில் பங்கேற்பவர்களின் இரத்தத்தில் உள்ள மூலக்கூறின் அளவை அறிந்துக் கொள்வதின் மூலம் புதிய இந்த நோய்களினால் ஏற்படும் நீரிழிவு விழித்திரை பாதிப்புக்கும் அதிகரித்த இரத்தத்தில் [illegible] மூலக்கூறின் அளவுக்கும் உள்ள தொடர்பை அறியலாம்.



6 நீங்கள் பங்கேற்க வேண்டுமானால் என்ன செய்ய வேண்டும்

நீங்கள் இந்த ஆய்வின் பங்கேற்கும் பட்சத்தில்

இந்த ஆய்வுக்கு தொடர்புடைய சில கேள்விகள்

[illegible] வழக்கமாக செய்யப்படும்

5

பரிசோதனைகள் செய்யப்படும், இரவு உணவுக்கு பிறகு ஏதும் சாப்பிடாமல் அடுத்த நாள் காலை வந்து இரத்த பரிசோதனை செய்யப்படும் அதில் இரத்தம் உள்ள மூலக் கூறின் அளவை அறிந்து கொள்வது. இரத்த அழுத்தத்தின் அளவையும், உங்களின் உயரம் மற்றும் எடையையும் அளவிடப்படும்.

மேற்கண்ட சிகிச்சை தேவைப்படும் என்றால் தேவைக்கு ஏற்ப இரத்தப் பரிசோதனை மற்றும் கண் பரிசோதனை மீண்டும் செய்யப்படும்.

நீங்கள் பங்கேற்பதனால் ஏதாவது பாதிப்பு / ஆபத்து ஏற்படுமா

உங்கள் கண் நோய் சம்மந்தப்பட்ட வழக்கமான பரிசோதனைக்கு இரத்தத்தில் உள்ள மூலக்கூறின் அளவை அறிந்து கொள்ள செய்யப்படும், இரத்த பரிசோதனை மட்டும் போதுமானது. நீங்கள் இந்த ஆய்வில் பங்கேற்பதனால் எந்தவித பாதிப்பும் ஏற்படாது என்று நம்புகிறோம். ஏதாவது பக்க விளைவுகள் ஏற்படும் பட்சத்தில் உங்களுக்கு இலவசமாக சிகிச்சை அளிக்கப்படும். பண இழப்பீடு

[illegible]

நீங்கள் பணம் ஏதும் செலுத்த வேண்டுமா ?

உங்களுடைய வழக்கமான சிகிச்சைக்கு உண்டான தொகையை மட்டும் செலுத்தினால் போதும். இரத்தத்தில் உள்ள நோய்க்கூறின் அளவை அறிவதற்கு தேவையான பரிசோதனை இலவசமாக செய்யப்படும்.

நீங்கள் பங்கேற்பதினால் என்ன நன்மைகள் உண்டு ?

1. நீங்கள் பங்கேற்பதினால் உங்கள் இரத்தத்தில் உள்ள நோய்க்கூறின் அளவு அதிகமாக இருக்கும் பட்சத்தில் உங்கள் கண் வளர்ச்சிக்கு சிகிச்சை செய்ய இது ஒரு முக்கிய பங்கை வகிக்கும்.

௨. உங்கள் இரத்தத்தில் உள்ள நோய்க்கூறின் அளவில் அசாதாரண நிலையில் இருக்கும் பட்சத்தில் மேற்கொள சிகிச்சை நிபுணர்களைக் கொண்டு சிகிச்சை அளிக்கப்பட

௩. ஆரம்ப நிலையிலேயே இது போன்ற அசாதாரண இரத்த அளவுகளுக்கு சிகிச்சை அளிப்பதன் மூலம் பக்கவாதம் மற்றும் மாரடைப்பு நோய்களை தடுக்கல

௪. நீங்கள் பங்கேற்பதினால் என்ன [illegible]

5)

இதனால் மற்றவர்களுக்கு என்ன நன்மை ?

இந்த ஆய்வின் முடிவுகளால் எதிர்கால நோயாளிகளுக்கு மருத்துவ அறிவு, நோய் தடுப்பு மற்றும் சிகிச்சையில் உள்ள முன்னேற்றம் பற்றிய அடிப்படை நன்மைகளை வழங்க / அதிகரிக்க முடியும். இந்த ஆய்வு மூலம் இந்த நோய் குறித்து கூடுதலான தகவல்களை பெறலாம்.

எதிர்காலத்தில் நீரிழிவு நோயாளிக்கு இந்த நோயை தடுக்கவும், நோயின் வளர்ச்சியை தாமதப்படுத்துவதன் மூலம் மிகவும் சிக்கலான பக்கவாதம் மற்றும் மாரடைப்பு நோயினை தடுப்பது குறித்த உத்திகளை அறிந்து கொள்ள உதவும்.

நீங்கள் பங்கேற்காமல் இருக்க முடியுமா ?

இந்த ஆய்வில் பங்கேற்பது என்பது உங்களுடைய விருப்பத்தின் / தன்னார்வத்தின் பேரில் தான் என்றும், எந்த நேரத்திலும் விலகிக் கொள்ள வாய்ப்பு உண்டு என்றும், அப்படி விலகிக் கொள்ளும் பட்சத்தில் [illegible] சிகிச்சை

உங்கள் மருத்துவரிடம் தொடர்ந்து சிகிச்சை பெற்றுக் கொள்ளலாம். இதனால் உங்களின் எந்த நன்மையும் பாதிக்கப்படாது.

இந்த ஆய்வின் முடிவுகள் மருத்துவ இதழில் வெளியிடப்படும். ஆனால் உங்களைக் குறித்த விபரங்கள் தெரிவிக்கப்படாது. இந்த ஆய்வில் உங்களைப் பற்றிய விபரங்களை ஆய்வுக் குழுவினர்கள் உங்கள் அனுமதியின்றி பயன்படுத்திக் கொள்ளலாம். எனவே, நீங்கள் இந்த ஆய்வில் பங்கேற்க முடிவு செய்ய வேண்டும்.

மேற்கொண்ட விளக்கங்களுக்கு டாக்டர் பிரபா குப்தா அல்லது டாக்டர் ஷீலா சுகன் ஜான் மற்றும் டாக்டர் தீபா ஜான் அவர்களை 0416- 2281201 என்ற தொலைபேசியிலும் drprabhagupta@gmail.com என்ற இணையதளத்திலும் தொடர்பு கொள்ளலாம்.

நீரிழிவு விழித்திரை நோய் மற்றும் ஹோமோசிஸ்டீன்
(எச் ஏ ஆர் டி)
(HARD)

ஒப்புதல் படிவம்

ஆய்வு எண் :

பங்கேற்பாளரின் முதல் எழுத்து :

பங்கேற்பாளரின் பெயர் :

பிறந்ததேதி / வயது :

(i) ________ அன்று எனக்கு கொடுக்கப்பட்ட தகவல் தாளை படித்து மேலும் எனக்கு ஏற்பட்ட சந்தேகங்களை கேட்க வாய்ப்பு கொடுக்கப்பட்டது.

(ii) இந்த ஆய்வில் பங்கேற்பது என்னுடைய முழு விருப்பத்துடன், தன்னார்வத்தின் பேரில் என்றும், எந்த நேரத்திலும் நிபந்தனையின்றி விலகிக் கொள்ளலாம் என்றும், இதனால் எனக்கு வழங்கப்படும் மருத்துவ சிகிச்சை மற்றும் என்னுடைய சட்ட உரிமைகள் பாதிக்காது என்றும் அறிவேன்.

(iii) இந்த ஆய்வை நடத்தும் ஆய்வாளர்கள் மற்றும் [illegible] உடனில் பணியாற்றுபவர்கள், நன்னெறிக்

④

சுகாதார விவரங்களை என் அனுமதியின்றி இந்த ஆய்வுக்கும் வேறு சில ஆராய்ச்சிக்கும் நான் இந்த ஆய்வில் விலகினாலும் பயன்படுத்திக் கொள்ளலாம் என்றும், என்னைக் இந்த அடையாளங்களை யாருக்கும் வெளிப்படுத்தப்பட மாட்டாது என்றும் அறிவேன்.

(iv) இந்த ஆய்வின் விவரங்கள் மற்றும் முடிவுகளை அறிவியல் பயன்பாட்டிற்கு மட்டுமே பயன்படுத்தப்ப வதற்கு ஒப்புதல் அளிக்கிறேன்.

(v) இந்த ஆய்வில் பங்கேற்க சம்மதிக்கிறேன்.

சுயபூர்வமாக ஒத்துக் கொள்ளப்பட்ட
பிரதிநிதி கையொப்பம் / கைரேகை பதிவு

தேதி :
கையெழுத்திடுபவரின் பெயர் :
கையொப்பம் :

ஆய்வாளரின் கையொப்பம் :
தேதி :
ஆய்வாளரின் பெயர் :
சாட்சியின் கையொப்பம் :

நீரிழிவு விழித்திரை மற்றும் [illegible]

(HARD)

தகவல் பிரதி - நோயாளர்கள்

நீங்கள் இந்த ஆய்வில் பங்கேற்க அழைக்கப்படுகிறீர்கள். உங்களுக்கு கொடுக்கப்பட்ட ஆவணத்தில் உள்ள தகவல்கள், இந்த ஆய்வில் நீங்கள் பங்கேற்க உதவுமா? அல்லது வேண்டாமா என முடிவு செய்ய ஏதுவாய் இருக்கும். மற்றும் இந்த ஆய்வைக் குறித்த கேள்விகள் (அ) சந்தேகங்கள் ஏதாவது இருப்பின் கேட்க தயங்க வேண்டாம்.

நீரிழிவு விழித்திரை நோய் என்பது கண்ணில் உள்ள விழித்திரையின் அல்லது ரெட்டினா (கண்ணின் பின்புறம் உள் நரம்பில்) உள்ள இரத்த நாளங்களில் ஏற்படும் பாதிப்பாகும். இது நீரிழிவு நோயினால் ஏற்படும் பாதிப்பை பொறுத்து மிதமான நிலையிலிருந்து (பாதிப்பில்லாத இரத்த நாளங்கள்) மோசமான நிலைக்கு (பாதிப்பை ஏற்படுத்தும் இரத்த நாளங்கள்) முன்னேறும்.

புதிய இரத்த நாளங்களினால் பாதிக்கப்பட்டவரின் விழித்திரையில் ரத்தக்கசிவு ஏற்படும் மற்றும் விழித்தி விலகிவிடும். எனவே இதன் முடிவு மோசமான பார்வை

(2)

ஆய்வாளரின் பார்வை இருப்பதற்கு முக்கியமானதா காரணமாகும்.

உடல் இருதய அடைப்பு மற்றும் இரத்தத்தில் அதிக அளவு கொழுப்பு போன்ற பல வியாதிகளுக்கு ஆரம்பநிலை உண்டுபண்ணும் காரணிகளாக உள்ளது. இவை குறைவால் இரத்தத்தில் அதிகரித்துள்ள மூலக்கூறு அளவும் காரணமாக இருக்கலாம் என்று சந்தேகிக்கப்படுகிறது/யூகிக்கப்படுகிறது. இரத்தத்தில் உள்ள இந்த மூலக்கூறு அதிகமாக இடம் பெறும் பாதிப்பு மற்றும் உடல்பருமன் போன்ற நோய்கள் ஏற்பட காரணமாகும்.

இந்த ஆய்வில் பங்கேற்பவர்கள் இரத்தத்தில் உள்ள மூலக்கூறின் அளவை அறிந்துக் கொள்வதின் மூலம் புதிய இரத்த நாளங்களினால் ஏற்படும் நீரிழிவு, விழித்திரை பாதிப்புகளின் அதிகரித்த இரத்தத்தில் சேர்க்கப்படும் மூலக்கூறின் அளவுடன் உள்ள தொடர்பை அறியலாம்.

② நீங்கள் பங்கேற்க வேண்டுமானால் என்ன செய்ய வேண்டும்

நீங்கள் இந்த ஆய்வில் பங்கேற்கும் பட்சத்தில் இந்த ஆய்வுக்கு தொடர்புடைய சில கேள்விகள் கேட்கப்படும். உங்களுக்கு வழக்கமாக செய்யப்படும்

பரிசோதனைகள் செய்யப்பட்டு, இரவு உணவுக்கு பிறகு ஏதும் சாப்பிடாமல் அடுத்த நாள் காலை வந்து இரத்த பரிசோதனை செய்யப்படு அதில் இரத்தம் உள்ள மூலக் கூறின் அளவை அறிந்து கொள்ளவும் இரத்த அழுத்தத்தின் அளவையும். உங்களின் உயரம் மற்றும் எடையையும் அளவிடப்படும்.

மேற்கண்ட சிகிச்சை தேவைப்படும் என்றால் தேவைக்கு ஏற்ப இரத்தப் பரிசோதனை மற்றும் கண் பரிசோதனை மீண்டும் செய்யப்படும்.

நீங்கள் பங்கேற்பதனால் ஏதாவது பாதிப்பு / ஆபத்து ஏற்பட

உங்கள் கண் நோய் சம்மந்தப்பட்ட வழக்கமான பரிசோதனைக்கு இரத்தத்தில் உள்ள மூலக்கூறின் அளவை அறிந்து கொள்ள செய்யப்படும், இரத்த பரிசோதனை மட்டும் போதுமானது. நீங்கள் இந்த ஆய்வில் பங்கேற்பதனால் எந்தவித பாதிப்பும் ஏற்படாது என்று நம்புகிறோம். ஏதாவது பக்க விளைவுகள் ஏற்படும் பட்சத்தில் உங்களுக்கு இலவசமாக சிகிச்சை அளிக்கப்படும். பண இழப்பீடு ஏதும் தர இயலாது.

நீங்கள் பணம் ஏதும் செலுத்த வேண்டுமா?

உங்களுடைய வழக்கமான சிகிச்சைக்கு உண்டான தொகையை மட்டும் செலுத்தினால் போதும். இரத்தத்தில் உள்ள மூலக்கூறின் அளவை அறிவதற்கு தேவையான பரிசோதனை இலவசமாக செய்யப்படும்.

நீங்கள் பங்கேற்பதினால் என்ன நன்மைகள் உண்டு?

நீங்கள் பங்கேற்பதினால் உங்கள் இரத்தத்தில் உள்ள மூலக்கூறின் அளவு அதிகமாக இருக்கும் பட்சத்தில் உங்கள் கண் வளர்ச்சிக்கு சிகிச்சை செய்ய இது ஒரு முக்கிய பங்கை வகிக்கும்.

உங்கள் இரத்தத்தில் உள்ள மூலக்கூறின் அளவில் அசாதாரண நிலையில் இருக்கும் பட்சத்தில் மேற்கொண் சிகிச்சை நிபுணர்களைக் கொண்டு சிகிச்சை அளிக்கப்படு

2. ஆரம்ப நிலையிலேயே இது போன்ற அசாதாரண இரத்த அளவுகளுக்கு சிகிச்சை அளிப்பதன் மூலம் பக்கவாதம் மற்றும் மாரடைப்பு நோய்களை தடுக்கல

[illegible] பங்கேற்பதினால் என்ன நன்மைகள் உண்டு

இதனால் மற்றவர்களுக்கு என்ன நன்மை ?

இந்த ஆய்வின் முடிவுகளால் எதிர்கால நோயாளிகளுக்கு மருத்துவ அறிவு, நோய் தடுப்பு மற்றும் சிகிச்சையில் உள்ள முன்னேற்றம் பற்றிய அடிப்படை நன்மைகளை வழங்க / அறிவிக்க முடியும். இந்த ஆய்வு மூலம் இந்த நோய் இருந்து விடுதலை தீர்வுகளை பெறலாம்

எதிர்காலத்தில் நீரிழிவு நோயாளிக்கு இந்த நோயை தடுக்கவும், நோயின் வளர்ச்சியை தாமதப்படுத்துவதன் மூலம் மிகவும் சிக்கலான பக்கவாதம் மற்றும் மாரடைப்பு நோயினை தடுப்பது இந்த உத்திகளை அறிந்து கொள்ள உதவும்.

நீங்கள் பங்கேற்காமல் இருக்க முடியுமா?

இந்த ஆய்வில் பங்கேற்பது என்பது உங்களுடைய விருப்பத்தின் / தன்னார்வத்தின் பேரில் தான் என்றும், எந்த நேரத்திலும் விலகிக் கொள்ள வாய்ப்பு உண்டு என்றும், அப்படி விலகிக் கொள்ளும் பட்சத்தில் உங்களுக்கு வழக்கமாக அளிக்கப்படும் சிகிச்சையில்

⑥

உங்கள் மருத்துவரிடம் தொடர்ந்து சிகிச்சை பெற்றுக் கொள்ளலாம். இதனால் உங்களின் எந்த நன்மையும் பாதிக்கப்படாது.

இந்த ஆய்வின் முடிவுகள் மருத்துவ இதழில் வெளியிடப்படும். ஆனால் உங்களைக் குறித்த விவரங்கள் தெரிவிக்கப்படாது. இந்த ஆய்வில் உங்களைப் பற்றிய விவரங்களை ஆய்வுக் குழுவினர்கள் உங்கள் அனுமதியின்றி பயன்படுத்திக் கொள்ளலாம். எனவே, நீங்கள் இந்த ஆய்வில் பங்கேற்க முடிவு செய்ய வேண்டும்.

மேற்கொண்ட விளக்கங்களுக்கு டாக்டர் பிரபா குப்தா அல்லது டாக்டர் ஸ்ரீஜா சுதன் ஆனந்த் மற்றும் டாக்டர் தீபா ஜான் அவர்களை Ph - 22812201 என்ற தொலைபேசியிலும் drprabhagupta@gmail.com என்ற இணையதளத்திலும் தொடர்பு கொள்ளலாம்.

நீரிழிவு சிறுநீரக நோய் மற்றும் இரத்தசோகையுள்ளோர்
(எச் ஏ ஆர் டி)
(HARD)

ஒப்புதல் படிவம்

ஆய்வு எண் :

பங்கேற்பாளரின் முதல் எழுத்து :

பங்கேற்பாளரின் பெயர் :

பிறந்ததேதி / வயது :

(i) ________ அன்று எனக்கு கொடுக்கப்பட்ட தகவல் தாளை படித்து மேலும் எனக்கு ஏற்பட்ட சந்தேகங்களை கேட்க வாய்ப்பு கொடுக்கப்பட்டது.

(ii) இந்த ஆய்வில் பங்கேற்பது என்னுடைய முழு விருப்பத்தில், தன்னார்வத்தின் பேரில் என்றும், எந்த நேரத்திலும் நிபந்தனையின்றி விலகிக் கொள்ளலாம் என்றும், இதனால் எனக்கு வழங்கப்படும் மருத்துவ சிகிச்சை மற்றும் என்னுடைய சட்ட உரிமைகள் பாதிக்காது என்றும் அறிவேன்.

(i) இந்த ஆய்வை நடத்தும் ஆய்வாளர்கள் மற்றும் அவர் சார்பில் பணியாற்றுபவர்கள், நன்னெறிக்

சுகாதார விவரங்களை என் அனுமதியின்றி இந்த ஆய்வுக்கும் வேறு சில ஆராய்ச்சிக்கும் நான் இந்த ஆய்வில் விலகினாலும் பயன்படுத்திக் கொள்ளலாம் என்றும், என்னைக் குறித்த அடையாளங்களை யாருக்கும் வெளிப்படுத்தப்பட மாட்டாது என்றும் அறிவேன்.

(iv) இந்த ஆய்வின் விவரங்கள் மற்றும் முடிவுகளை அறிவியல் பயன்பாட்டிற்கு மட்டுமே பயன்படுத்தப்படுவதற்கு ஒப்புதல் அளிக்கிறேன்.

(v) இந்த ஆய்வில் பங்கேற்க சம்மதிக்கிறேன்.

சுயபூர்வமாக ஏற்றுக் கொள்ளப்பட்ட
பிரதிநிதி கையொப்பம் / கைரேகை பதிவு

தேதி :
கையெழுத்திடுபவரின் பெயர் :
கையொப்பம் :

ஆய்வாளரின் கையொப்பம் :
தேதி :
ஆய்வாளரின் பெயர் :

[illegible]

APPENDIX 6

ABBREVATION

DM - Diabetes Mellitus

PDR - Proliferative Diabetic Retinopathy

DR - Diabetic Retinopathy

T1DM – Type 1 Diabetes Mellitus

T2DM – Type 2 Diabetes Mellitus

Hcy - Homocysteine

AC - Fasting blood sugar

PC - Post-prandial blood sugar

HbA1c - Glycosylated Hemoglobin

Hb - Hemoglobin

WHO - World Health Organisation

CURES - Chennai Urban Rural Epidemiology Study

NPDR - Non Proliferative Diabetic Retinopathy

tHcy - Total homocysteine

SAM – S-Adenosyl Methionine

MTHF - Methyltetrahydrofolate

BMI - Body Mass Index

NVD - Neovascularization at Disc

NVE - Neovascularization Elsewhere

CKD - Chronic Kidney Disease

eGFR - Estimated Glomerular Filtration Rate

VEGF - Vascular Endothelial Growth Factor

IRMA - Intra Retinal Microvascular Abnormalities

DME - Diabetic Macular Edema

CSME – Clinically Significant Macular Edema

MTHFR - Methyltetrahydrofolate Reductase

ETDRS - Early Treatment Diabetic Retinopathy Study

DVT - Deep Vein Thrombosis

CAD - Coronary Artery Disease

CVA - Cerebro Vascular Accidents

RVO - Retinal Vein Occlusion

RAO - Retinal Artery Occlusion

HPCL - High Performance Liquid Chromatography

SD - Standard Deviation

CN - Control

LDL- Low Density Lipoprotein

RBCs - Red Blood Cells

BP – Blood Pressure

H – Hemorrhage

VB – Venous Beading

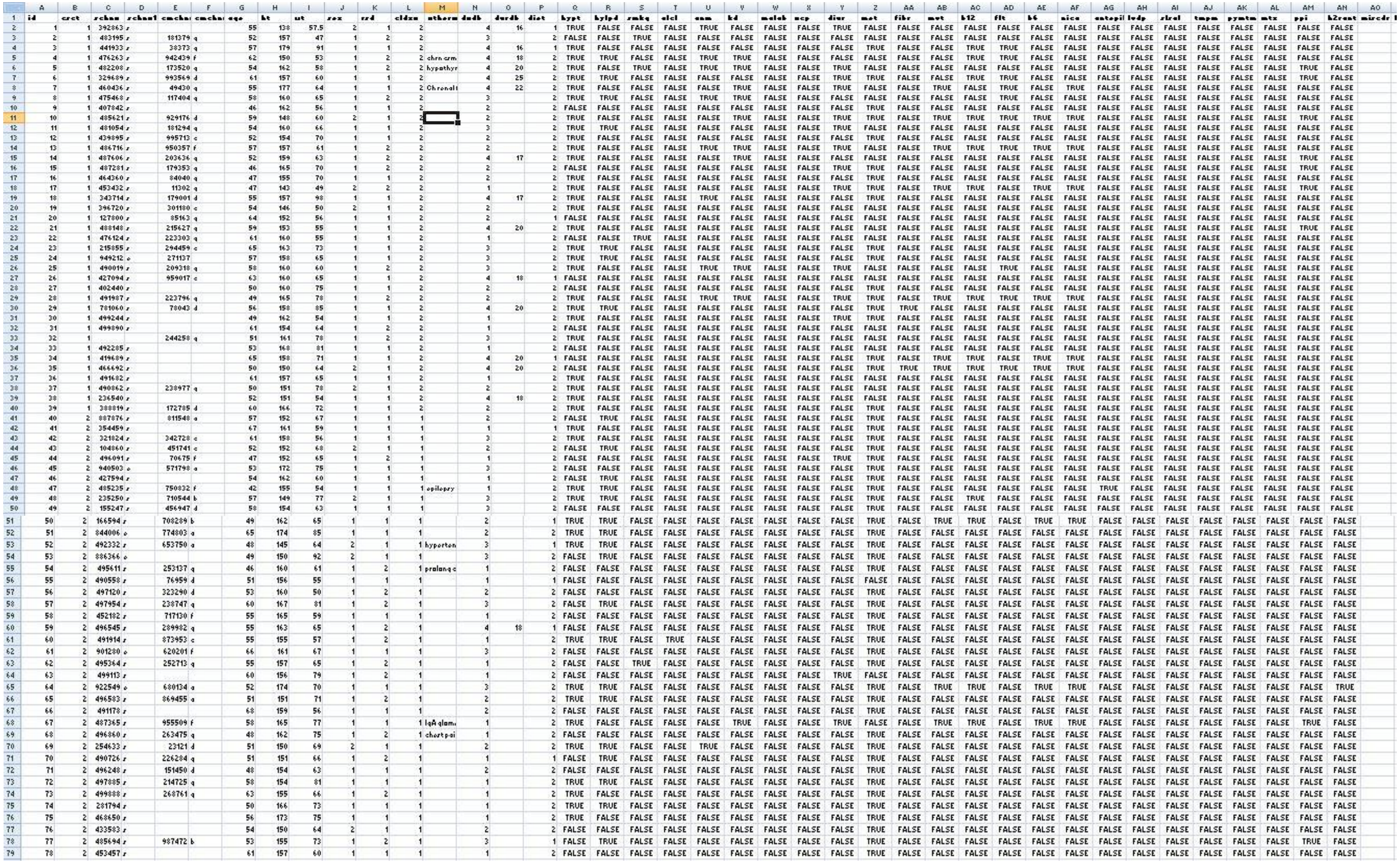
Ma – Microaneurysm

ROS – Reactive oxygen species

CMC – Christian Medical College

IOR – Interquartile range

APPENDIX 7: MASTER DATA SHEET



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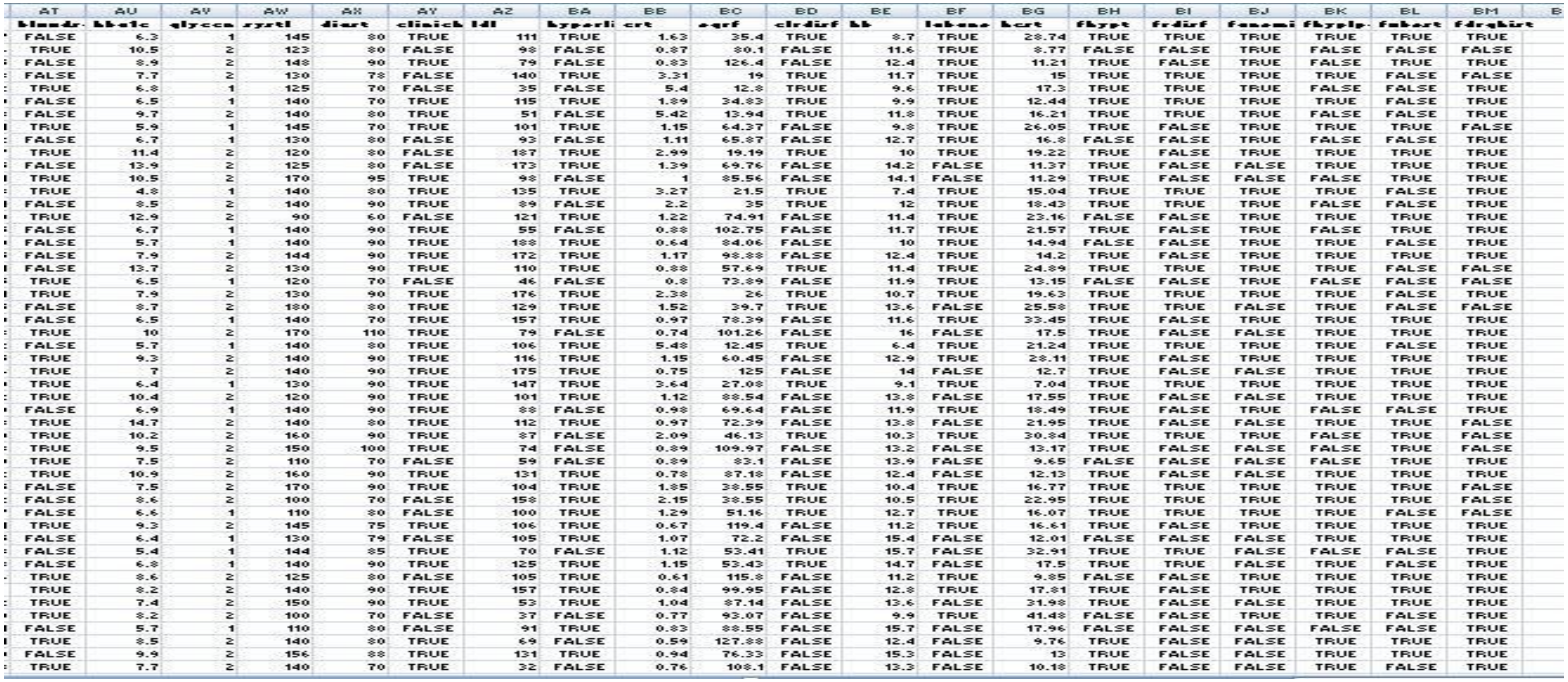
79	78	2	453457	r			61	157	60	1	1	1		1		2	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	TRUE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	



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FALSE	7.7	2	130	78	FALSE	140	TRUE	3.31	19	TRUE	11.7	TRUE	15	TRUE	TRUE	TRUE	TRUE	FALSE	FALSE
TRUE	6.8	1	125	70	FALSE	35	FALSE	5.4	12.8	TRUE	9.6	TRUE	17.3	TRUE	TRUE	TRUE	FALSE	FALSE	TRUE
FALSE	6.5	1	140	70	TRUE	115	TRUE	1.89	34.83	TRUE	9.9	TRUE	12.44	TRUE	TRUE	TRUE	TRUE	FALSE	TRUE
FALSE	9.7	2	140	80	TRUE	51	FALSE	5.42	13.94	TRUE	11.8	TRUE	16.21	TRUE	TRUE	TRUE	FALSE	FALSE	TRUE
TRUE	5.9	1	145	70	TRUE	101	TRUE	1.15	64.37	FALSE	9.8	TRUE	26.05	TRUE	FALSE	TRUE	TRUE	TRUE	FALSE
FALSE	6.7	1	130	80	FALSE	93	FALSE	1.11	65.87	FALSE	12.7	TRUE	16.8	FALSE	FALSE	TRUE	FALSE	FALSE	TRUE
TRUE	11.4	2	120	80	FALSE	187	TRUE	2.99	19.19	TRUE	10	TRUE	19.22	TRUE	FALSE	TRUE	TRUE	TRUE	TRUE
FALSE	13.9	2	125	80	FALSE	173	TRUE	1.39	69.76	FALSE	14.2	FALSE	11.37	TRUE	FALSE	FALSE	TRUE	TRUE	TRUE
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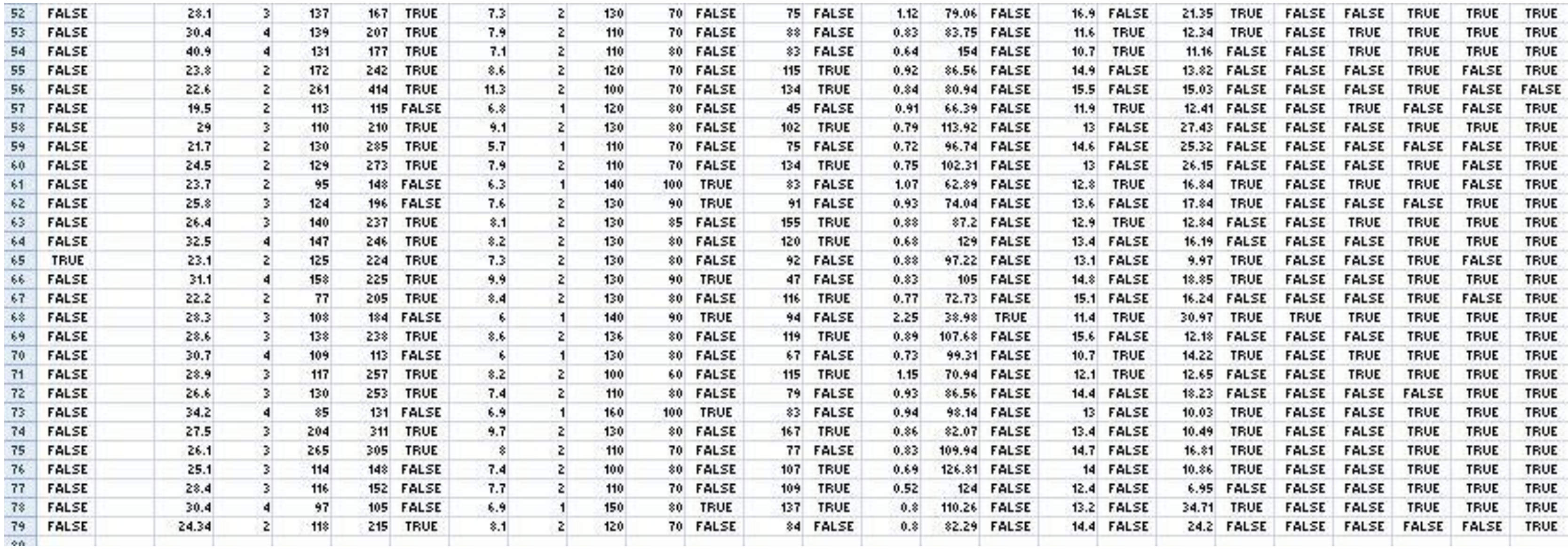

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55	FALSE		23.8	2	172	242	TRUE	8.6	2	120	70	FALSE	115	TRUE	0.92	86.56	FALSE	14.9	FALSE	13.82	FALSE	FALSE	FALSE	TRUE	FALSE	TRUE
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57	FALSE		19.5	2	113	115	FALSE	6.8	1	120	80	FALSE	45	FALSE	0.91	66.39	FALSE	11.9	TRUE	12.41	FALSE	FALSE	TRUE	FALSE	FALSE	TRUE
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